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Carter and the test of energy

FOR the first time since the Arab oil embargo, the United States Government seems ready to consider adopting a comprehensive energy policy, one which involves efforts to curb the nation's profligate consumption as well as attempts to step up domestic energy supplies. Such a policy is long overdue, but it will severely test the will and the political skills of the new Carter Administration to put it into practice.

Although both Mr Nixon and Mr Ford paid a good deal of lip service to the need for energy conservation during their terms in office, their energy policies consisted essentially of throwing large sums of money into the supply side of the energy equation. Indeed, when Mr Nixon launched his infamous Project Independence, he even insisted on making absurd claims that American technology would free the United States from relying on imported oil to meet its energy needs by 1980. The demand side of the equation was badly neglected, perhaps for understandable reasons.

The United States has grown up with cheap power, with the ethic that bigger means better, and with the philosophy that the government has little right to meddle in the workings of the so-called free market. All that would have to be changed if a serious attempt is made to curtail energy demand. Mr Nixon, who was already under siege when the oil embargo struck, probably lacked the political ability to pursue effective conservation policies—in any case, he didn't make much of an effort. And Mr Ford suffered the handicaps of being an unelected President who belonged to a different political party from the one which controlled the Congress and who had an abiding faith in the free market.

President Carter may be different. His campaign rhetoric was peppered with promises to develop an energy policy, and he has repeatedly warned that it will require considerable 'sacrifice' from the American people. He also comes to office riding a tide of goodwill and—perhaps most important—this year's extraordinary winter has provided a more vivid argument for conservation than a thousand Presidential statements. If Carter doesn't manage to put an energy policy into effect, it would be a serious indictment of his Administration's political ability.

So far, Carter's efforts are promising. The first major instalment of his energy policy came last week when he proposed legislation to consolidate most of the federal government's sprawling energy bureaucracies into one Cabinet-level department under the direction of James Schlesinger, former head of the Atomic Energy Commission, the Central Intelligence Agency and the Department of Defense. Though the proposed arrangement is eminently sensible, the bill would clump together agencies and pro-

grammes which are now handled by different Congressional committees. Some jurisdictional disputes may therefore delay passage of the Bill, but approval is nevertheless anticipated.

Once in place, the Department of Energy should be in a better position to plan both conservation and supply policies. That's where the second instalment of Carter's energy policy comes in. He has promised to unveil a comprehensive energy plan on 20 April, with particular emphasis on conservation. A preview of at least some of the elements of that policy was provided last month when Carter sent Congress a number of revisions to the budget proposals which Mr Ford left behind.

As far as research and development are concerned, Carter's policy will concentrate on efforts likely to produce results in the relatively near term. Thus, long term efforts such as the breeder reactor programme, nuclear fusion efforts, and the development of large scale solar power plants, would be cut back and the money reprogrammed into energy conservation and into efforts to get already-developed technologies, such as solar heaters, into the market-place. The justification for such moves is that it will probably be more difficult to commercialise new energy technologies than to develop them, and government incentives are required to feed the growth of, say, a solar energy industry. Those arguments are likely to find favour in Congress.

As for curtailing energy use, the Administration can pursue only a limited number of technological fixes, such as forcing the automobile industry to make more efficient cars and encouraging the use of battery-driven vehicles. But the most effective energy conservation measure would be to increase energy prices through the use of federal energy prices through the use of federal energy taxes.

Such a move would, however, be very difficult to get through the Congress and it would also have to be coupled with a system for returning the tax revenues back into the economy, preferably in a manner which would mitigate the burden on low income groups. It is estimated that every cent of tax on a gallon of gasoline would raise about \$1,000 million in revenue; a large tax could have a depressing effect on the economy. One idea which is being floated is to return some of the tax revenues into energy-saving measures such as income tax credits for home insulation, construction of mass transit systems and so on. In any case, the Carter Administration has already begun to leak the fact that it is considering raising energy prices in order to prepare the American public for the inevitability of a long overdue move away from the present artificially low prices of energy. □



Between the devil and the deep blue sea

Robert W. Cahn, Dean of the School of Applied Sciences at the University of Sussex, offers this comment on the prospect for fusion power

In the anguished debates on the future, if any, of nuclear fission power, various recognisable strands emerge among the opposition. Some pin their faith on the improved technological exploitation of familiar fossil sources, principally coal; others would drive us headlong into the solar economy, often conceived in terms of 'small is beautiful' and a rural life of log-cabin simplicity; yet others accept the need for high technology on a very large scale if the sun is to yield domestic warmth, electricity and liquid fuel on an adequate scale.

All three groups, however, have one characteristic in common. When faced by hard statistical or technological criticisms, they declare that we need only keep going for a few decades and then the boundless energy "from seawater"—that is, nuclear fusion—will become a reality and all our worries will be over. Although they do clearly recognise that the method is not altogether simple, even the most resolute proclaimers of radioactive doom seem to have convinced themselves that fusion power will be gentle and on a homely scale, and that radioactive pollution will be a thing of the past when dirty fission gives way to clean fusion. Probably the fact that fusion takes place in the sun and that the sun is the emblem of cleanliness and of life itself, has something to do with this touching faith. We are all to some degree subject to the ad-man's technique of fallacious association.

There is no dearth of popularisations of this very difficult and complex topic, but they all concentrate on two aspects of fusion—how to confine the plasma, and how to heat it to ignition temperature. Even within these limits, they tend to concentrate on scientific principles and not on engineering realities, whereas the current criticism of nuclear fission concentrates on real and alleged engineering weaknesses. Nuclear fusion has progressed far enough now towards the design of a self-sustaining reactor to make it both possible and necessary to see the problems in realistic terms. The plasma confiners—which indeed have a ferociously difficult task—until recently have hogged all the attention, to the neglect of the problems of energy balance (achieving a positive net energy output), container strength and fatigue damage, lithium supply, reactor cooling, injection of gas into the plasma without quenching it, and especially the radiation damage inside the reactor itself.

That this imbalance is now being repaired is primarily due to the efforts of the Energy Research and Develop-

ment Administration (ERDA), which has complete control of fusion research in the United States. ERDA has prepared a detailed R&D plan extending to 1990, and in outline to the end of the century. ERDA's achievement is to have set out this programme without committing itself to any particular design of reactor, and yet to have identified the central problems which will have to be faced irrespective of the design. To do this, ERDA's predecessor in 1967 invented the concept of the 'reference design'. This is an outline based on scientific principles, as presently understood, set out in such a way as to identify, as quantitatively as possible, the whole range of engineering problems that will need to be mastered. Such a reference design is particularly illuminating when applied to the doughnut-shaped Tokamak, which in purely scientific terms is today probably the most hopeful contender among the rival approaches.

On the basis of such a reference design, the problem of securing the deuterium (by electrolysis of water?) and tritium from a metre-thick breeding blanket of molten lithium surrounding the first wall of a doughnut-shaped plasma device, can be analysed, not least in terms of lithium availability; lithium is a scarce element. One day it will also be necessary to include in the energy balance equation not only the energy cost of extracting tritium from irradiated lithium but also the electricity costs of electrolysis water to extract deuterium, a calculation which never seems to be reported.

Probably the most alarming engineering problem is that of radiation damage. In the much-criticised fission reactor, neutrons, gamma rays and fission particles remain inside the reactor volume, and indeed the fission products remain inside the nuclear fuel

itself. In a Tokamak, all the neutrons, moving with a kinetic energy of some 14 MeV, beat upon the first wall: the scale of this bombardment dwarfs anything in a fission reactor. The wall can be sputtered away, especially by the novel hazard of 'chunky sputtering' which involves the physical removal of thousands of atoms at a time.

A great deal of research is now in progress on this group of problems in the United States (some also in Britain), and considerable advances have been made in improving the radiation resistance of high-strength refractory alloys. But it is certainly an error to regard fusion reactors as generating little radioactivity: there will be a great deal of induced radioactivity, though not from fission products, and it will be just as crucial as in the case of a fission reactor to contain and seal off this radioactivity from the outer world. (It seems likely that this hazard will not be long-lived). Apart from this, there will be just the same hazards of pinholes developing in inaccessible locations as have been found with prototype fission-breeder reactors, leading to deterioration in cooling efficiency or vacuum quality.

Fusion may prove to be a practical source of energy—the new issue is now as much in the hands of the engineers and metallurgists as of the plasma physicists. If it comes, it will be an immeasurable boon but it will also bring with it problems of pollution, energy imbalance during construction, resource depletion, sabotage and potential stoppage such as we find with every other source of energy, including solar techniques such as the large-scale fermentation of timber to make liquid fuel.

For Blake, energy was a mark of the divine. Milton knew better: Satan has energy too. Whether the external flames are fed by coal, oil, uranium or deuterium is a question which must tax the physical theologians of the future. □

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Europe's nuclear debate (1)

Austria: a case study

Helmut Hirsch and Helga Nowotny assess
Austria's nuclear energy information campaign

NUCLEAR energy has become a topic of interest in Austria much later than in other countries, so her politicians and scientists have been able to study the development of the issue for some time. It has also come at a time of social stability, when even a small disturbance would not go unnoticed. This, and the manner in which the authorities control and institutionalise conflicts, help to make Austria an interesting case-study. For unlike the experience of other western countries, Austria's nationwide nuclear debate was initiated by the government. It organised the *Informationskampagne Kernenergie* (nuclear energy information campaign), and from being a topic important only to a few environmentalists and dwellers near proposed reactor sites, the subject became a national concern drawing more and more citizens into action groups.

The first steps were taken in July 1975. The Federal Chancellor's Office was responsible for the organisation, and a committee of civil servants, chaired by the head of the Energy Section of the Ministry for Trade, Commerce and Industry, Dr W. Frank, took over the scientific preparation. An exhaustive catalogue of questions was compiled, divided into well-defined subtopics embracing economy, safety, environmental and biomedical questions. In accordance with frequent practice and to avoid suspicions of manipulation, the cooperation of nuclear energy critics was sought, among them the Austrian Nobel-prize winner Professor Konrad Lorenz, and Doz. B. Lötsch, the head of the Viennese Ludwig Boltzmann-Institute for Environmental Sciences.

Each of the subtopics was to be dealt with by a group of experts, consisting of critics and promoters of nuclear energy in equal numbers (about three of each). This pro-con classification later proved to be an over-simplification: many scientists don't fit into either category, many more belong clearly to one side but refuse to admit it. Preliminary discussions would clarify as many points as possible, while the open problems would be left for the public debates. Finally, a report was to be produced containing relevant information, answers to questions on which consensus was reached and recommendations for further research. Although the organisers believed scientific controversies arose simply because there was not enough data (unless they con-

cerned an issue that wasn't a real scientific problem), this later proved a rather naive conception as controversies also exist about which data can be regarded as relevant and about the appropriate framework for their interpretation.

Emphasis on independence

Strong emphasis was placed on the independence of the experts. People employed by electrical utilities, the nuclear industry, or concerned with the licensing of nuclear power plants participated only in internal debates as consultants. Civil servants were also excluded as experts from the various ministries because they would have to contribute in subsequent parliamentary debates. Thus, the government could remain non-committal, not being responsible for what independent experts choose to say or write. For the same reason, politicians from the governing Socialist Party participated little; their frequent absence from the debate was often criticised.

Promoters of nuclear energy felt that the campaign would emphasise the disagreement between experts and only increase public fear and suspicion. But most critics failed to appreciate the genuine attempt to avoid the errors made in other European countries. Hence the emphasis on sub-groups working on different topics—too often discussions had remained superficial and disorganised; and the attempt to balance the expert groups between pro and con—too often 'information' had proved to be only state-sponsored propaganda.

The government took the final decision to initiate the campaign on 17 February 1976. As there were ten subtopics, ten moderators for the expert-groups, mostly university professors, were selected by Dr Frank. As every expert has a personal opinion or a professional bias on the subject, it was impossible to choose strictly neutral moderators. Nuclear physicists and economists specialising in energy problems tended to be pro-nuclear, whereas the rest were critical or at least suspicious. Most felt ill at ease in their positions.

The internal debates, organised and conducted by the moderators, started in June 1976. No questions were excluded from the public debate because it was impossible to define clearly solved and unsolved problems.

To prepare the public, the Federal

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Chancellor Kreisky: his office organised campaign

Press Service issued a booklet providing basic information, *Nuclear Energy—A Problem of Our Time*. Although controversial issues were reserved for the subsequent debates, disagreement among the authors (mainly the moderators) was considerable and many passages were tentative and non-committal. Nevertheless, both critics and promoters of nuclear energy voiced negative and positive opinions: one group of critics requested that all copies of the booklet be destroyed, while another wanted 2,000 copies for local distribution and complained about the small number printed (15,000).

The experts and organisers, at least the pro-nuclear ones, imagined that the public would listen eagerly and trustingly to the specialists' lectures in spite of strong fears about nuclear energy. The majority of anti-nuclear experts saw the audience as a concerned population with legitimate claims. The possibility of changes in the concept of the campaign as a result of effective, well-organised public action was ignored by the organisers as impossible to incorporate within the chosen framework. This indicated little flexibility in the organisation and an unwillingness to accept active public participation.

The main features of public reaction to the campaign became evident during the first public discussion in Vienna on 14 October 1976. There was strong resentment at the idea of listening passively to a panel discussion and only being allowed to submit factual questions in written form; the public demanded open discussion and it soon became apparent that the rules would be impossible to enforce. Massive distrust was expressed in the campaign: there was some surprise in the first debate when it became apparent that three of the panel experts were nuclear energy critics as opposed to mere propagandists.

Lack of consideration for the political viewpoint was another source

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of provocation to the public: while general problems concerning nuclear power were being discussed, Austria's first plant was nearing completion. The decision to build had been taken long ago and the construction proceeded throughout the campaign. Pressure was exerted to stop the plant going into operation and a resolution was passed to this end. Finally, the concept of having separate topics for each evening, in different towns, was criticised; although the theme of the first, general social and economic problems, was sufficiently broad, some of the subsequent ones were of little interest to the public which was anxious to discuss the more crucial issues.

In spite of the criticisms expressed during the first debate, plans for the next discussion in Linz remained unaltered; it was to be even more strictly controlled from the platform. The reaction was especially vehement, as the topic of the evening, energy policy, did not seem relevant to the people living near the proposed site of the second nuclear power plant. In addition, no outspoken critic was among the panel. Consequently, with almost unanimous consent, about fifty organised critics took over the panel and continued the meeting with their own chairman.

To reduce dissatisfaction with the division into different topics, it was decided to reinforce the panels with 'consultants', mainly experts in the biological and medical fields, who could be called upon if necessary. The next four debates proceeded uneventfully, even when two of the panels, dealing with economic problems, showed a strong pro-nuclear bias. The public discussions became less rigidly controlled and organisers and chairmen held preparatory talks with local opposition groups before each meeting, giving the latter the right to read longer statements or resolutions if they respected the rules of the debate.

The precarious balance achieved by these conciliatory measures broke at the seventh debate, in Vienna, on 27 January 1977. The hall could not seat all the people who arrived but after massive pressure short of violence, everyone was finally allowed in. The first panel statement, made in a provokingly patronising way by a promoter of nuclear energy, was halted by interruptions and a recognised critic among the panelists elected a new chairman. Thereafter, the discussion concentrated on how to stop the construction of Austria's first nuclear power plant.

The following two meetings, held in small provincial towns, were less dominated by the critics; these were comparatively peaceful, although the

main criticisms were upheld. The final debate in Vienna, due on 24 March, will probably be controlled entirely by the opponents of nuclear power.

Coverage of the campaign by the mass media has been poor, being limited to superficial accounts of the arguments presented by the experts or the more spectacular actions of the protesters. Opponents have developed the theory that the government is deliberately hindering publicity in order to avoid raising public concern. Although radio and television are not state-controlled, it is indeed debatable whether sufficient efforts have been made to ensure adequate coverage.

At present, plans for the second phase of the campaign are in progress. Representatives of opposition groups, electrical utilities, the nuclear industry, as well as employers' and employees' organisations, will be invited to four symposia. These, to be held in May and June 1977, will be devoted to a discussion of the reports compiled by the ten expert groups. The results, together with a summary provided by the government, will be submitted to parliament, to serve as background for a subsequent decision on nuclear energy, and then published.

From a preliminary evaluation, certain points emerge:

- The organisers exercised considerable control, through their choice of experts, on the political credibility of the debate. But they had no influence over public reaction.

- Considerable differences emerged between public and internal debates. While the latter usually took place in a friendly atmosphere in which disagreements were respected by colleagues, opponents and proponents were extremely conscious of their respective roles in the public debates.

- The public was highly distrustful of expert panels set up by the authorities. There were accusations that experts were being paid by the nuclear lobby and that the government was staging a mere puppet show with political decisions having already been taken behind closed doors.

- The public reaction manifested 'scientific populism' and resentment of establishment science. Dissenting scientists quickly became celebrated as heroes. Although opposition groups sometimes displayed a moving willingness to delve into scientific details, anything outside their pre-conceived notions was unacceptable to them. What they demanded was a 'people's science'—a body of knowledge controllable by them.

- Coverage by the mass media was mostly determined by factors unrelated to the information campaign. It tended to dismiss the protest movement as

Traditionally, a large percentage of Austria's electricity requirement has been met by hydropower. The growing energy demands of the country's economy, however, finally led it to consider the nuclear option. In 1971 construction of the first nuclear power station began near Zwentendorf in Lower Austria. It is being built by the West Germany company Kraftwerke Union, although Austrian firms supply know-how and a considerable number of components. The company owning it is half state-owned and half-owned by the utilities in the federal provinces. The station should be supplying electricity to the grid by March 1978.

Public concern grew when talk began of a greater commitment to nuclear energy. The construction of a 1,300 MW plant was originally scheduled to begin in 1976; another was planned for the early 1980s. In the spring of 1975, groups of concerned citizens in Upper Austria—near the proposed site of the second plant—collected over 60,000 signatures in a local petition against the plans.

With nuclear energy a much debated issue in other countries and producing violent confrontations, with the recession (felt only slightly in Austria) reducing the need for greater electricity generating capacity, and with an election planned for the autumn of 1975 creating additional pressure on the government, all decisions concerning future commitments to nuclear power were postponed. Work on the planning of the second and third plants was suspended; construction of only the first plant proceeded normally. Later the government declared that the alternative to nuclear power would be increased use of (mainly imported) fossil fuels or imported electricity. In February 1977, responding to questions submitted by the opposition in parliament, the government pointed out that this alternative was economically feasible, although parliament would have to decide whether it was desirable. This decision is expected at the end of 1977. Of the three parties represented in the Austrian parliament, the governing Socialist Party is divided over the issue, but has a pro-nuclear bias, and the People's Party is pro-nuclear but highly critical of the way the government handles the problem: the smallest, the Liberal Party, is decidedly anti-nuclear.

In the summer of 1976, the nuclear energy critics founded a nationwide federation outside existing parties incorporating eight local groups in all but one of the federal provinces. The number has since increased to at least twelve.

extremist or based on irrational fears; opposition newspapers automatically wrote in a negative way about the campaign. Only in Vorarlberg, where the population had been made aware by opposition to a planned Swiss reactor close to the border, did the local movement receive strong press support.

- The authorities had not considered the political context sufficiently: the public was expected to consist of 'neutral citizens' only, to whom information would be offered as a public service. This neutral citizen, however, either doesn't exist, or is not sufficiently interested to attend the meetings. □

Europe's nuclear debate (2)

Germany: growing acrimony

Werner Gries looks at the controversy over the use of nuclear power in West Germany

THE row in West Germany over the peaceful use of nuclear energy has grown increasingly fierce over the past few months. It reached its latest climax on 19 February with the siege of the nuclear power station at Brokdorf, where scores of people were detained by the police after a clash with militant demonstrators who tried to occupy the power station site. A similar demonstration last November, also at Brokdorf, was also violent. Meetings held all over the country to discuss the necessity for nuclear energy at all have produced clashes between supporters and opponents of nuclear power. The press, radio and television have busied themselves conducting detailed investigation into the pros and cons of the issue.

The result of all this activity, in some eyes at least, is little result at all. In spite of the wave of anti-nuclear demonstrations, a recent opinion poll indicates that a slight majority of the population is in favour of the construction of nuclear power stations. In an all-party resolution last year, the Bundestag (parliament) declared itself unanimously in favour of the continued use of nuclear energy so that West Germany's future energy supplies could be guaranteed; the Ministers President of the Länder (provinces of the federal republic) followed suit. Such agreement at government level, though, has not prevented various branches of the Social Democratic party calling for a halt to the building of nuclear stations; in some cases they have organised joint demonstrations with local people to protest against new plants. All political parties are involved in the debate, and each has in its ranks both supporters and opponents of nuclear power—the governing coalition of Social Democrats and Free Democrats displays more disagreement than the opposition Christian Democrats and Christian Socialists.

Federal complications

Additional complications have sprung from the country's federal framework. Individual Länder, for example, are responsible for authorising nuclear power stations in their own areas. Several local courts have granted injunctions to stop the building of new plants because of protests by private citizens. In Schleswig-Holstein, a court ruled that work on the Brokdorf nuclear power station could not start until a way of disposing of the waste had been agreed on, and in mid-February a

court in Rhineland-Palatinate banned further work on the Müllheim-Karllich station near Coblenz because of technical errors in the construction. The government of North Rhine-Westphalia has banned the building of all new power stations within its area until a method of waste disposal is agreed. Some sites have been brought to a standstill, others have experienced partial stoppages; at some sites it has not even been possible to begin the construction work.

One of the major obstacles to have arisen to the expanded use of nuclear power has focused on the reprocessing and storage of radioactive waste. Although the importance of the problem was recognised when West Germany first began using nuclear energy, the necessary political measures to deal with it were not set in motion. What is more, after 1980 it may not be possible for West German fuel elements to be reprocessed in France or Great Britain. In both countries there is growing opposition to the expansion of reprocessing capacity, especially for foreign fuel. Germany's present contracts run out in 1979.

Faced with this problem, the West German government has resolved to authorise the building of new power stations only if the safe disposal of fuel elements can be guaranteed from the start. It has plans for a *entsorgungszentrum* ('service centre'), which could reprocess the fuel elements from all West German nuclear power plants and store the radioactive waste products in salt deposits for hundreds of years. The site chosen for the centre is Niedersachsen, but a fierce argument has flared up locally which may have far reaching effects on the construction of nuclear power stations in other parts of West Germany. Yet unless the service centre can be established, the expansion of West Germany's nuclear power capacity will be halted. Government leaders and the opposition therefore feel that they must jointly take steps to ensure that a centre is built.

Overtaking Britain

At the end of 1976, 13 nuclear stations were in operation in West Germany, with a total output of 6,500 MW. West Germany has overtaken Britain in terms of nuclear power capacity and is the leading user of nuclear energy in the EEC. A further 17 nuclear power stations are proposed, some of which are already under construction. There

were plans for 50 by 1985, with a total capacity of approximately 45,000 MW, but because of the recent public protests the construction of some planned nuclear power stations has been postponed in certain areas, and this figure now seems utopian. Experts now believe that about another 25 stations will be operational by 1985, with a total capacity of approximately 22,000 MW.

The government believes that the country faces an electricity shortage in the 1980s and in spite of the current difficulties, large scale research is going on to develop more advanced reactors. Some DM2,000 million each year are allocated for nuclear energy research, mainly for developing a fast breeder and a high temperature reactor. For many years there has been bitter argument over the necessity for the fast breeder, and this has grown even more bitter during the past few months. It remains doubtful whether an acceptable site can be found, and the special security problems make it more unlikely that the public will ever accept it.

Compared to the internal difficulties, discussion of Germany's role in international trade in nuclear technology has been comparatively limited. The controversial deal with Brazil, providing eight nuclear power stations, a reprocessing plant and an enrichment plant by 1990, was originally welcomed by all parties in the Bundestag, and the government and opposition still agree that it is necessary. The Carter Administration is objecting to the reprocessing and enrichment plants because it fears they will provide Brazil with the raw materials for an atomic bomb, and has endeavoured to pressure Brazil too. With reports now of US hold-ups in supplies of enriched uranium to West Germany, nothing is certain, but the general public seems to accept that the West German-Brazilian treaty should be adhered to in its original form. Their more immediate concern is with the domestic implications of a domestic programme. And that concern is likely to be fuelled still further in the future. □

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Demo at Brokdorf

UNITED NATIONS

Preparing for 1979

A Special Correspondent reports on preparations for the United Nations Conference on Science and Technology

THE appointment in January of Mr Joao Frank da Costa of Brazil as Secretary-General of the United Nations Conference on Science and Technology, which is scheduled for 1979, has enabled preparations to get under way. Sitting as the Preparatory Committee for the Conference, the 54-nation Committee on Science and Technology for Development gave a mixed reception to Mr da Costa's views on the organisation and timing of the conference preparations. He secured agreement, however, on several vital points, the most important of which is perhaps that he should have as free a hand as possible in the selection of the conference secretariat.

Kurt Waldheim, the UN Secretary-General, had stated that the post of Deputy Secretary-General of the Conference should be held by Dr Klaus-Heinrich Standke, the Director of the Office of Science and Technology in New York, and that his staff should form the core of the conference secretariat. From the first, Mr da Costa made it clear that he could not accept this arrangement, especially as Dr Standke was expected to double up two responsible jobs from now until the conference. No deputy as such has been appointed; the Executive Secretary of the Conference, Mr Hans Einhaus, who has lately represented the Office of Science and Technology in Geneva, is holding this position in an acting capacity.

Da Costa was less successful in persuading the committee of the practicality of some of his other ideas, such as the suggestion that each member state should produce three preparatory documents for the conference. If previous performance is anything to go by, many governments will have difficulty in producing a single really useful paper, as now requested, although the option to produce more remains open for those who can. Regional papers will also be prepared by the several UN Economic Commissions. With those from the other members of the UN system and from major organisations outside the UN, these will make up a considerable amount of documentation. To offset this, the number of meetings, seminars and so on suggested for the preparatory period will be reduced; the suggestion that the conference secretariat should be regionalised was also dropped.

No precise agenda is expected until this time next year, but the general objectives of the conference are already well defined. They include: the adoption of concrete decisions on ways of applying science and technology, as a strategy aimed at economic and social development "within a time frame"; strengthening the technological capacity of the developing countries; the adoption of means for the utilisation of science and technology in solving the problems of development; and the provision of "instruments of cooperation" to developing countries, for solving socio-economic problems that cannot be solved by individual action.

In general, it does seem as though the agenda will be action-oriented; one thing da Costa stressed was the need to avoid the "ritual complications"

inherent in the preparations for all such conferences—the ritual of producing documents and of setting-up institutions do not of themselves solve problems, but are only tools to that end. In general, the objective of the conference will be to assess and re-define the role that science and technology has to play in development. Moreover, as seen by da Costa the preparations themselves can help towards the definition of a unified policy for science and technology within the United Nations system.

One other point on which da Costa did not get his way was in the location of the conference secretariat. He had asked that it be set up in Geneva, but this was overruled, the majority of delegations preferring New York, where they can oversee, if not interfere in, the conference preparations. Many regard this as unfortunate. One of the reasons for the success of the Environment Conference in 1972 was the insistence of Maurice Strong, as Secretary-General, that his headquarters should be in Geneva, away from the UN bureaucracy.

The New York siting could also prejudice really close collaboration on the part of the other members of the system, although their involvement has been one of the avowed intentions of those who suggested the conference in the first place. Geneva is central to these organisations—UNESCO in Paris, UNIDO and IAEA in Vienna, FAO in Rome and, in Geneva itself, WHO, WMO, UNCTAD and ILO. It is in these organisations that the bulk of international knowledge and experience in applying science and technology lies, and the possible economies of having the secretariat in New York may well be offset by a loss of their close collaboration and good will. □

CANADA

Support for alternatives

David Spurgeon reports from Ottawa on Canada's interest in alternative energy sources

CANADA, a country once thought to possess fabulous natural gas and petroleum supplies, is now carefully assessing its renewable energy resources. The government has recently increased the funds available for research on tidal power, wind power, biomass and solar energy. And the

whole subject was highlighted at a recent meeting of the Parliamentary and Scientific Committee sponsored by SCITEC, the Association of the Scientific, Engineering and Technological Community of Canada. It was the second meeting of the committee, which was formed to bring parliamentarians and scientists together, and in spite of scepticism that this could be done, both meetings were well attended.

One of the most interesting energy potentials discussed was the Bay of

Fundy's tidal power. Separating the two Maritime provinces of Nova Scotia and New Brunswick, the Bay of Fundy possesses one of the world's largest tide ranges. A. N. Karas, assistant director of planning for the Electrical Engineering Branch of the National Energy Board, said that although the last detailed study in 1968 concluded that Fundy power projects were uneconomic (albeit feasible), significant increases in power generation costs had helped the two provincial governments and the federal government to agree in 1975 to re-evaluate such schemes.

The conclusion was that the combined market for a 7,000 MW tidal

power plant—in the Maritimes, in the contiguous areas of Canada (mainly Quebec), and in the Northeastern United States—would by 1990 be more than ten times the capacity of the plant, or about 77,000 MW, and that such a plant could be brought into operation by that time. A survey indicated that the anticipated peak load in the Maritimes would grow from 3,216 MW in 1980 to almost 23,000 MW by the year 2010. For Quebec, the peak demand would be 18,682 MW in 1980 and 133,000 MW in 2010. And in the Northeastern United States, the figures would be 18,000 MW in 1980 and 87,500 MW in 2010. Thus the full output of the plant could be used.

The energy displaced by a tidal power plant in the Maritimes, furthermore, would be predominantly high cost oil-fired and some coal-fired energy, the latter mainly in Nova Scotia. "In 1990 tidal energy would displace about 4,000 GWh of oil-fired generation and 500 GWh of coal-fired generation," Mr Karas said. "This is equivalent to about an annual savings of 6 million barrels of oil and 300,000 tons of coal. By the year 2010 the corresponding annual fuel savings have been calculated to be about 9 million barrels of oil and 600,000 tons of coal." To serve the tidal plant's export markets, extra high voltage transmission lines (735 or 750 KV) would be required.

The scheme has considerable appeal to residents of Nova Scotia, who have been hard hit by the recent increases in oil prices. Residents of that province pay some of the highest prices in the country for home heating. The president of the Nova Scotia Power Corporation, L. F. Kirkpatrick, was quoted in the *Globe and Mail* as saying that, based on recent construction cost estimates of between \$1,300 million and \$1,700 million, power could be produced at one or other of the Bay of Fundy sites for between 18 and 29 mills per kilowatt. Currently, oil-produced electricity costs almost 20 mills.

Dr E. P. Cockshutt, co-ordinator of the National Research Council of Canada's Energy Project, gave a cautious but positive forecast for the utilisation of solar energy in Canada. There is sufficient solar energy available in all the reasonably populated regions of Canada to make it a useful resource, he said, giving the lie to those who have played it down in the past. But there were unique features of climate and geography that necessitated a distinctly Canadian programme.

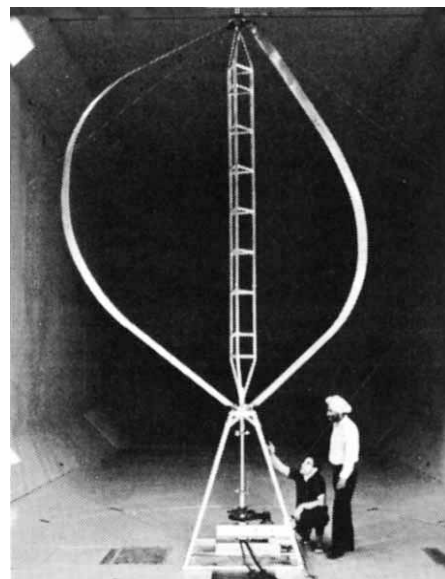
An attack on the technical problems of durability and cost was the most

important priority for the next two or three years, Dr Cockshutt said, noting that "popular enthusiasm for solar heating presently outruns the technical and commercial realities." Large-scale commercialisation of solar heating is at least five to ten years away, but it is expected eventually to become a billion-dollar-a-year industry, he said.

Total radiation outside the earth's atmosphere available to a properly-oriented receiver amounts to about $1,400 \text{ watts m}^{-2}$, and that actually received on a horizontal surface at ground level in most of Canada is about one-tenth that (150 watts m^{-2}) on a year-round average basis, Dr Cockshutt said. Even the sunniest parts of the world receive less than twice as much, or about 250 watts m^{-2} . And with properly inclined collectors, the difference would be reduced. But it is because of the extremes of seasonal variation that a Canadian solar energy programme must differ greatly from those in countries close to the equator. In addition, the reflective properties of snow-covered ground differ distinctly from those of vegetation.

Professor H. M. Lapp, of the University of Manitoba's Department of Agricultural Engineering, called biomass a major energy source for Canada in view of the magnitude of its production annually. "The energy available in animal waste, crop residues and forestry waste represents approximately 6.72, 2.74 and 9.46% of Canada's national energy consumption, or a combined biomass energy potential of 18.92% of Canada's national energy requirement," he said. "Such a significant component of Canada's national energy requirements available from renewable sources should be examined carefully for the feasibility of economically viable development." Considering wood alone, he said it had been estimated that ethanol/methanol fuel production potential from wood was about $2\frac{1}{2}$ times Canada's current annual consumption of motor gasoline and approximately 110% of its total gasoline plus fuel oil consumption.

The head of the NRC's low speed aerodynamics laboratory, R. J. Templin, estimated that, with average one- to two-mile spacing between megawatt-sized wind turbine power plants with a total height of about 100 m, the average wind power available over the whole of the country was about 130,000 MW (overall conversion efficiency 15%). If it was assumed that suitable sites were limited to areas within 150 km of an existing network, the available average power was reduced to about 60,000 MW—about twice Canada's national electricity consumption in winter months. Even without energy storage, "it appears that wind



Wind up: turbine in tunnel

energy constitutes a very large *theoretical* resource in Canada," he said. The main obstacles to large-scale wind energy development were the need to demonstrate long-life reliability and to reduce capital costs.

A number of small-scale wind power projects are currently in progress in Canada, including several at the Brace Research Institute, Montreal, and a rotor designed by Brace installed by Hydro Quebec's Research Institute for experiments on compressed air storage. The only large-scale project is the 200 kW vertical axis turbine now being erected by Hydro Quebec on the Magdalen Islands, one of the windiest sites in Canada. Several new versions of the turbine are under development at two Canadian companies with government funding assistance.

Only days before the SCITEC meeting, the federal government showed its interest in renewable energy resources by announcing that research in the area had been awarded the largest share of a \$10 million increase in federal funding for 1977-78 for energy research. A Renewable Energy Resource Branch has been established within the Department of Energy, Mines and Resources, and a National Advisory Committee on Conservation and Renewable Energy is being created.

There was a \$4.4 million increase for renewable energy research (including solar, wind, biomass and the use of heat pumps); energy conservation was allotted \$3.7 million more; coal, heavy oils and oil sands research was increased \$1.5 million; and transportation and energy transmission received an increase of \$460,000. The increases will bring total federal energy R&D funding to approximately \$138 million in the next fiscal year. □

VIKING

Give us a call, says NASA

Alex Dorozynski reports from Paris on the Viking symposium held there last week

NASA's next Mars package will be launched from Cape Kennedy in January 1984. It will carry a roving laboratory capable of covering at least 100 km and perhaps as much as 1,000 km of the planet. The orbiting satellite will contain 'penetrators', to be implanted at and to transmit information from selected locations, as well as remote sensing and transmission equipment. Lodged several metres into the Martian soils, the penetrators will record seismic activity and will try to determine the chemical composition of Martian soil below the surface. No attempt will be made to bring soil samples back to Earth; the earliest date for a rational sample return mission, rather than an indiscriminate 'grab sample' attempt, is 1988.

This schedule is not an official one, but it sums up the consensus of opinion at last week's symposium. The symposium involved senior scientific and management personnel of the Viking programme, the European Space Agency and the French National Centre of Space Studies. The Viking team will now tour several European countries, including the Soviet Union, to inform interested scientists of the latest Viking results and to emphasise that NASA planetary exploration programmes are open to international participation.

According to the Viking chief project scientist, Dr Gerald A. Soffes, no part of the schedule is in the approved stage. But the outlook is favourable indeed. By any standards—science, technology, public appeal—Viking has been an outstanding success, and data is still pouring in from the two landers and the two orbiters. These will all be kept at work for at least another full year. One single orbiter may continue to relay information from both landers, while the other will be switched over to different orbits in order to roam the surface of the red planet with its remote sensing equipment.

President Carter has recently proposed an increase in NASA 'study money' for 1978 (the increase awaits Congressional approval) but the agency does not seem to want to go to Mars with another Viking just for the sake of it; rather it wishes to exploit the massive flow of data to prepare the next Viking generation. The 1979 'astronomical window' to Mars seems too close for this, so the project for

early 1984, even though it is not optimal from the energy viewpoint, has been adopted as a goal by the study team and this is already at work.

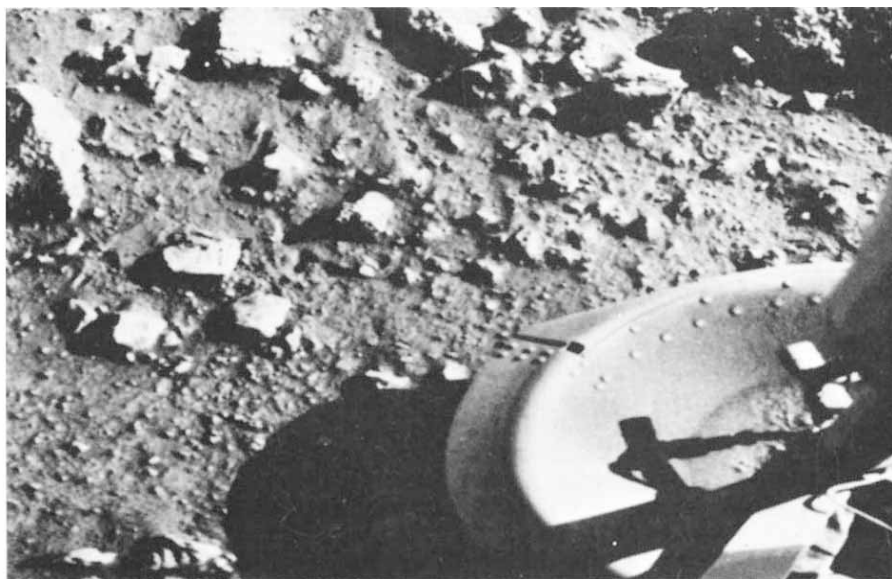
Dr Soffes and his colleagues made a point of inviting non-American guest scientists to participate in the study of Viking data and the elaboration of future missions. According to A. Thomas Young, the Viking missions' operations manager, participation would include the elaboration of scientific experiments, instrumental design, and perhaps even the loading on board of an experimental package—provided of course it fits into the overall package. Dr Young suggested that the first proposals be made to the NASA by mid-May this year, so that planning of the still-hypothetical 1984 mission could start early in October.

Meanwhile all Viking data is being made available to interested scientists. Major reports have been published in three issues of *Science*; these have not apparently been found sufficiently detailed and complete by some specialists, so a thorough review is now being prepared, including most recent data, for publication in the *Journal of Geophysical Research* in September.

Dr Soffes also hopes for an international Viking conference similar to the one that followed the Mariner mission, but, he says, "it is not easy to put together or to know when it should happen." In the meantime limited discussions on specific aspects of Viking results will be held during other meetings. These will take place, for example, at the lunar samples meeting in Houston in March, the meeting in Kyoto in June of the International Biological Society

for the Study of the Origin of Life, and the pulsar meeting in Tel Aviv, also in June. Scientists are also being encouraged to contact NASA directly concerning aspects of Viking in which they are particularly interested.

New data is coming in so rapidly that publications cannot keep up without delays of six months or more, and the participants in the Viking symposium at the *Palais de la Découverte* in Paris were given more than enough to whet their appetites. On the menu: the Mars quake, registered during the primary mission, which took place some 50 km away from the landing site, and was measured between 2 and 3 on the Richter scale; the first close range photograph of Phobos, one of Mars's small moons; the decreasing atmospheric pressure, now increasing again as winter approaches in the northern hemisphere; the verification of Einstein's general theory of relativity; the phenomenon of Mars' dust storms and the unusual erosion patterns that have been photographed and still remain a puzzle. Finally, of course, there are the biology experiments, which Dr Leslie Orgel, Head of the Viking molecular analysis team, described as the most positively negative, adding that in fact they had given no answer; Viking had landed in one of the "most desolate positions on Mars". Other sites will be explored during the next mission; but Mars conditions, or at least some of them, are now being re-created in the laboratory to find out whether earthly life forms can survive in them. As other sites may have conditions entirely different from the ones at the Viking landing sites, there is still hope for life. "We may have found that where it's red, it's dead" quipped Dr Orgel; but Mars is red all over. □



Back to 1976: a view from Mars

USA

● Congress has already begun to whittle down the hefty budget increase for basic research proposed by President Ford in his valedictory budget. A key House subcommittee last week substantially reduced the budget request for the National Science Foundation (NSF), and recommended some shifts in priority among NSF's programmes. Though the sub-committee's actions are likely to be revised as the NSF budget bill moves through the rest of the Congressional budget process, the proposed reductions at least sound a warning that Congress isn't going to rubber stamp the research and development proposals this year.

Ford has recommended that basic research in general be given a budgetary shot in the arm to help restore some of the inflationary losses incurred during the past few years. It has been estimated that real support for basic research in the United States shrank by about 20% between 1967 and 1975, and Ford accordingly proposed that federal support be increased next year by more than 9%—well above the anticipated 6% inflation rate. NSF's budget, in particular, would have gone up by more than 12% under Ford's proposals. Those recommendations were left unchanged by President Carter when he submitted his budget revisions to Congress late last month.

Last week, however, the House subcommittee on Science, Research and Development, which is usually friendly toward NSF, approved an authorisation bill for the foundation which would provide an increase of about 9%—enough to keep NSF a step ahead of inflation, but substantially less than the Ford budget proposal. The subcommittee, moreover, has shifted funds within NSF's total budget in such a way that the programme of Research Applied to National Needs (RANN) would bear the brunt of the reductions, while the foundation's education support programmes would be given more money than Ford had requested.

The RANN programme would be given about the same overall budget as it received this year, but the subcommittee has recommended that a sharply increased proportion of RANN funds should be spent on earthquake research. Other RANN projects would consequently be cut back. Outside those two areas, the subcommittee has essentially recommended that NSF's programmes be given cost-of-living increases.

The subcommittee's action is only

the first stage in a protracted budget process. The subcommittee bill will be taken up by the full Committee on Science and Technology this week before being sent to the House itself and, on the senate side, a subcommittee chaired by Senator Edward Kennedy has just completed hearings



on NSF's authorisation bill; it is expected to report its own version soon. Appropriations committees in both the House and Senate will then take up the measure.

It is thus likely that the subcommittee's bill will be modified considerably as it progresses through the rest of the Congressional mill, but the proposed reductions nevertheless suggest that NSF's budget may be in for a bumpy ride through Congress this year.

● Another distinguished scientist has been appointed to a top-level post in the Carter Administration. Donald Kennedy, professor of zoology at Stanford University, was last week named Commissioner of the Food and Drug Administration (FDA), a post which has been occupied by physicians for the past 12 years.

The appointment is something of a surprise since Kennedy has had little direct experience in the political minefield of drug regulation. FDA is constantly caught in a fierce crossfire between the drug industry, consumer advocates and Congressional critics. It has been chronically short of funds to carry out its mission or regulating the powerful food, drug, cosmetics and medical devices industries, and its own research enterprise is generally acknowledged to be relatively low in quality. Kennedy will clearly have his work cut out for him.

A neurophysiologist, Kennedy's political star has been rising rapidly in Washington in the past few years. Elected a member of the National Academy of Sciences in 1972 at the

age of 40, he was chairman of an Academy committee which last year produced a massive and widely-publicised report on pest control problems. Late last year he was appointed a senior consultant to the newly established Office of Science and Technology Policy (OSTP), a job which took up half his time and kept him shuttling back and forth between Washington and Stanford. While at OSTP, Kennedy is said to have played a major role in securing a substantial increase for agricultural research in President Ford's final budget. Otherwise, his experience in Washington politics has been limited.

● An international coalition of scientists, environmentalists, theologians and philosophers has been formed to seek a worldwide moratorium on most uses of recombinant DNA techniques and broader public discussion of the ethical and social implication of genetic engineering. The coalition, whose formation was announced at a press conference in Washington on Monday, includes among its sponsors Sir MacFarlane Burnett, an eminent Australian immunologist and Nobel Prize-winner. Other sponsors include Aurelio Peccei, founder of the Club of Rome, Lewis Mumford, author and philosopher, George Wald, and a number of environmentalist organisations and church leaders.

A statement endorsed by the group, which calls itself the Coalition for Responsible Genetic Research, notes that "genetic and molecular biological research is rapidly providing the basis for the practice of genetic engineering". It continues: "The continuation of this research without public understanding and approval, and in fact without a full comprehension of its potential by most of the involved scientists, poses a worldwide danger which is intensified by the fact that industrial investment in the developing genetic technology has already begun".

Citing possible health hazards from current research, including "the spread of cancer or the creation of new diseases", the statement calls for a moratorium "on all research that would produce novel genetic combinations between distant organisms which have not been demonstrated to exchange genes in nature". The moratorium should stay in effect, the coalition argues, while the ethical implications of the research are examined and the dangers assessed in public discussions.

Colin Norman

View and review

Further, there has been extensive activity, centred on Colorado, to modify hail which accounts for \$700 million of damage in the United States every year. A strategy already adopted in the Soviet Union is to increase by a factor of up to a thousand the number of nuclei in the hail-producing part of a cumulonimbus cloud by launching shells or rockets packed with silver iodide which explode inside the cloud. The hope is that many small hailstones rather than a few large ones will fall. Soviet work has extended well beyond the research phase and annual savings running into tens of millions of roubles have been reported. Work in France, Switzerland and Kenya has yielded mixed results, again with problems of randomisation, but the National Hail Research Experiment initiated in 1971 in the United States has yet to bear fruit.

IN BRIEF

More trouble for dissidents

Seven Moscow Jewish activists, all members of the Voronel/Azbel' seminar and/or the Helsinki monitoring group, have had their homes searched unusually thoroughly by the police. The seven are the cyberneticist Aleksandr Lerner, Josif Beilin, a mathematician, Ida Nudel, an economist, and the engineers Vladimir Slepak, Anatolii Shcharanskii, Boris Chernobil'skii and Mikhail Kremen. An open letter in *Izvestiya* signed by Dr Senya Lipavskii, a former associate of these groups, accuses them of being financed by CIA funds. Lipavskii also claimed that during their protest hunger strike of March 1974, the sinologist Vitalii Rubin and the chemist David Azbel' did, in fact, eat regular meals. (Both Rubin and David Azbel' have since emigrated, Azbel' in 1974 and Rubin in 1976).

Dissident circles in Moscow associate

the new repressions with a recent article in *Pravda* urging political vigilance "as never before". In Warsaw, meanwhile, the latest batch of arrests of members of the Workers' Defence Committee has included the mathematician Jan Lytinski and Mieczyslaw Grudzinski, a computer engineer.

Argentina-Peru deal

Argentina's growing role in Latin America's development of nuclear power was given another boost at the end of last week when she signed an agreement with Peru to build a 10 MW research reactor there. The agreement, reportedly involving a \$50 million budget, covers design, equipment and training of local scientists. Argentina herself has a 300 MW reactor in operation, and a 600 MW reactor under construction. Recently she announced her

support for Brazil's efforts to maintain the controversial deal with West Germany, the original source of guidance for her own programme.

BNFL: another step

Plans submitted to Cumbria County Council by British Nuclear Fuels Ltd (BNFL) for expansion of existing Magnox reprocessing facilities and for facilities to develop its Harvest waste solidification process were, as expected, quickly accepted by the council's planning committee last week.

BNFL split into two its more controversial oxide fuel reprocessing plans. The part covering construction of the plant is now expected to be called in for a public inquiry. The other part, relating to the provision of storage facilities for incoming fuel, will, BNFL says, not prejudice any decision on the plant itself.

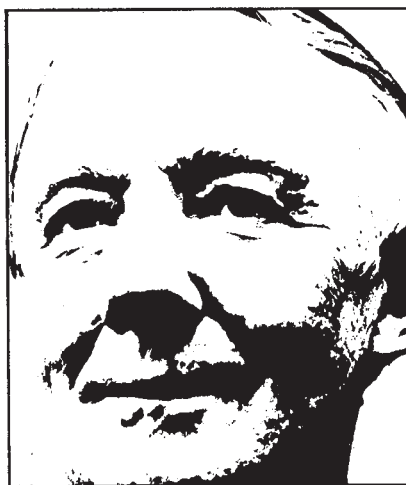
SCIENCE is being taught today in British universities, polytechnics and colleges in many different ways. We still have special honours degrees, where one subject is dealt with 'in depth', with the intention of producing specialists. It is generally agreed that brilliant students, particularly if they intend to follow a career in their speciality, may thus be well served. However, some departments now offer such a variety of optional courses that the result may be unrecognisable to the traditionally minded. Zoologists may never study a whole animal, botanists may know little about common plants. Even when such fashionable absurdities are avoided, many would agree that less gifted students, only able to scrape a pass, are better served by more broadly based instruction.

The 'interdisciplinary degree', however, is not new. Fifty years ago Part One of the Cambridge Natural Science Tripos (taken in three subjects), or the similar London University General Honours course were familiar examples; both entitled their holders to an honours BA or BSc. The degrees were considered suitable for the less able, or as a preliminary to specialisation in a single subject.

Today, there is a much wider choice. Modular degree courses may allow students to choose combinations of subject which appear illogical to conservative academics. Even when, as is true of most colleges, students are sensibly counselled, so that the subjects studied are reasonably compatible, those entering these courses

may have somewhat doubtful job-prospects, particularly serious at a time of graduate unemployment. It would be little consolation for a prospective chemist to be told that he is now better suited to enter "the

Grade expectations



KENNETH MELLANBY

upper reaches of the Civil Service, banks, the highest positions in industry" because he is now better educated than his contemporaries studying classics (if his course fits the criteria suggested by Sir Hermann Bondi, *Nature* 27 January 1977) when the Royal Institute of Chemistry finds itself unable to recognise his qualifications.

This was why the Council for Science and Technology Institutes,

which coordinates the work of the recognised bodies in Britain, held a meeting in London recently to discuss how multidisciplinary degrees could qualify their holders for admission. It was clear that all the institutes were anxious to find ways to allow these graduates to qualify, provided this did not lower their standards. Most give credit for those parts of the course which are relevant, and suggest how essential gaps may be filled. As all the institutes only elect graduates after several years of experience in their subject, the length and nature of this experience can be adjusted to overcome deficiencies. It is clearly important that all teaching departments should know, in advance, how new courses are likely to be viewed by the professional institutes, as well as by prospective employers.

Membership of professional institutes may become increasingly important, as these qualifications become the means by which scientists are recognised within the Common Market. It is also clear that the greater difficulty a candidate may have in being accepted the more valuable membership will be to him. The institutes must be fair and sympathetic in all cases, but must also be seen to be maintaining their standards. They must always remember Groucho Marx, whose applications to join an exclusive club were repeatedly blackballed. Eventually he was informed that he had been successful, upon which he asked "Who would want to join a club which admitted a bum like me?"

correspondence

The teratogenic effects of dioxin

SIR,—Alistair Hay (10 February) states that many pregnant women from the dioxin-contaminated district of Seveso have sought terminations outside Italy. Some will have to come to Britain: UK statistics should reveal whether relatively more abortions were performed on Italian women in 1976 than 1975.

In view of the highly teratogenic activity of dioxin in animals, and the total absence of information for our own species, I share Dr Hay's concern that no organised steps have been taken to examine aborted foetuses for possible malformations. I would urge that some code of practice be agreed, if possible for Europe as a whole but at least for Britain, to ensure that all foetuses aborted as a result of any suspected contamination of the environment should be examined by an experienced pathologist and compared with a matched control series. We may be too late this time: but Seveso is unlikely to be the last accident of its kind.

Yours faithfully,

ANNE McLAREN

MRC Mammalian Development Unit,
University College, London

Journal abbreviations

SIR,—I agree with Adrian Smith (10 February, page 492) that the saving in typesetting does not in itself justify the use of journal abbreviations. I suggest, however, that the real problem lies not in abbreviating but in establishing a correct and unambiguous title. Here the British Standard is of no help, but the *World List of Scientific Periodicals* is invaluable, for journals published up to 1960.

The examples of ambiguous abbreviation cited by Mr Smith do not come from the *World List*, where there is a clear distinction between *J. math. Phys.* and *J. Math. Phys.* Had they existed in 1960, *Industrie minérale* would have become *Industrie minér.*, while *Industrial Minerals* became *Ind. Miner.* Even in the annual supplements published since 1960, which have unfortunately abandoned the principles of the fourth edition of *World List*, *Ind. Miner. (Fr)* is not the same as *Ind. Miner.*

The *World List* gave a standard for journals with multilingual titles, and distinguished, if distinction be thought

necessary, between three journals called *Nature*.

Correct identification of current journals will eventually be possible by the use of International Standard Serial Numbers, though whether this will reduce the chore to authors and editors remains to be seen.

Yours faithfully,

J. E. M. HORNE

Freshwater Biological Association,
Windermere

Charged plumes

SIR,—Contrary to the report of Jennings and Jones on high electric fields from industrial stack plumes (November 18, 1976), the electrical effects in the atmosphere caused by electrostatic precipitators are well known. Those working in the field of atmospheric electricity are aware that plumes emanating from chimneys equipped with precipitators contain large numbers of charged particles which can be detected at a considerable distance from the chimney¹.

Much of the charge may be carried on solid particles which somehow have escaped capture on the precipitator surfaces. We think that some fraction of the charge is also carried by water molecules from the combustion products in the industrial operation and of course there will be some small ion generation from the breakdown of other gases near the high voltage portion of the precipitator.

We have measured effects of chimney plumes with equipment carried in a manned aeroplane^{2,3,4}. Among other results, we observed both the horizontal and vertical atmospheric electric field components up to 4,000 feet above the chimneys on calm days, when thermal convection of the plume was more pronounced than motion downwind. Rowland⁵ came across the plume phenomena in 1973 when making measurements in a clear air turbulence study.

Vonnegut *et al.*⁶ purposely generated clouds of charged oil droplets with a device similar to an electrostatic precipitator and reported extremely high local electric fields which caused point corona discharge currents and other high-field effects on bushes, structures, people, etc. He also levitated a charged cloud of water droplets below the oil cloud.

To the extent that charges generated in this manner (by chimney precipitators) can somehow become transferred onto high mobility carriers, the authors may be right in asserting that there is a possible contribution to the earth's charge balance.

We doubt that the low mobility, charged solid particles contribute directly, but the high mobility charges, which they may generate by pulling point discharges from earthed structures, might. The first result of this point discharge current in the atmosphere, of course, will be to help discharge the chimney plume; no doubt this is partly why the electrical effect of the plume is diminished as it travels away from the precipitator.

The observation of a possible hazard from sparking in the near vicinity of such plumes is well taken, and the cement and electrical power industries should be interested in further investigation into this likelihood.

Yours faithfully,

MAYNARD L. HILL

THEODORE R. WHYTE

ROGER O. WEISS

Applied Physics Laboratory,
Johns Hopkins University,
Laurel, Maryland 20810, USA

¹ Moore, A. D. *Electrostatics and Its Applications* 402 (John Wiley & Sons, N.Y., New York, 1973).

² Hill, M. L. & Whyte, T. R. "Investigations Related to the Use of Atmospheric Electric Fields for Aircraft and RPV Stabilisation," APL/JHU TG 1280, June, 1975.

³ Whyte, T. R. "Atmospheric Electric Field Research Program—Preliminary Report for April, May and June, 1975," APL Report BAF-75-07, July, 1975.

⁴ Hill, M. L., Weiss, R. O. & Whyte, T. R. "Investigation of the Use of Atmospheric Electric Fields as a Vertical Reference for Airborne Vehicles, Progress Report #3," APL Report BAF-76-20, September, 1976.

⁵ Rowland, J. R. "Airborne Measurements of the Atmospheric Electric Potential Gradient Associated with Haze, Smoke Plumes, and Clear Air Convection," APL Report MPD73U-032, 3 May, 1973.

⁶ Vonnegut, B., Smallman, C. R., Harris, C. K. & Sykes, W. G. "Technique for the introduction into the atmosphere of high concentrations of electrically charged aerosol particles," *Jour. of Atmospheric and Terrestrial Physics* 29, 781-792, 1967.

Bird at Pompeii

SIR,—T. E. Thompson (27 January, page 292) surely owes an apology to the anonymous artist of Pompeii. The little bird he portrayed sitting on a rock overlooking the sea is clearly a composite representation of all those passerine migrants of the type known to Americans as 'confusing fall warblers' which are still trapped and eaten on a vast scale all round the Mediterranean coastline. It is interesting that they are the only terrestrial delicacy considered worthy of inclusion in a picture of local specialties. They are of course those 'nightingales' whose tongues formed the favourite diet of the Roman emperors.

Yours faithfully,

W. R. P. BOURNE

Milltimber,
Aberdeen, UK

news and views

Unsolved problems in nuclear structure

from Otto Kofoed-Hansen

In preparing the balance sheet for any particular branch of science, much subjective argument is involved. So what follows is my very personal stock-taking of nuclear physics—where do we need more experimental information?

Where exactly is the yrast line?

Quoting Bohr and Mottelson (*Nuclear Structure II*, Benjamin, 1975) “the lowest level of given spin is referred to as an “yrast” level (Grover *Phys. Rev.* **157**, 832; 1967). The word appears to derive from the old Germanic *wor*, which developed into the English *weary* and the Nordic *orr* (modern Swedish *yr*, modern Danish *ør*), meaning dizzy. The form *yrast* is the naturally constructed superlative of *yr*”. (In other disciplines of physics the name “the lowest Regge trajectory” appears more natural.)

For a given nucleus composed of Z protons and N neutrons, (mass number $A=Z+N$) there exists a multitude of possible states. The states are characterised in their energy content and in sets of quantum numbers (such as spin, I , which characterises the angular momentum of the nucleus as a whole). For each spin value, I , there exists a lowest energy content below which no (N, Z) nucleus of this spin can be formed. This lowest energy limit is called the yrast line—at this line all the energy available is used to make the nuclear structure in question spin. For simple heavy nuclei there is also a maximum energy content beyond which the nucleus will fission; centrifugal forces will split it. Thus there exists for all NZ combinations and all values of I a region limited on one hand by fission and on the other hand by yrast (dizziness). Large groups of nuclear physicists are attempting to pin down these limits and to understand them in terms of nuclear models. The tools envisaged are heavy ion accelerators and all possible refinements in detector techniques. The aim is to create very large

spin states of representative combinations of N and Z and to watch the details of the decays of these states. The mere fact that N , Z and I can vary within a relatively wide range means that this is a vast region of research. The interpretation is forseen in terms of collective nuclear models, but bordering on those limits where every single nucleon in the nucleus carries as much oriented angular momentum as it can get away with. The next question thus follows quite naturally.

Where is the borderline between continuum descriptions and individual particle descriptions of nuclei?

By ‘individual particle description’, I understand a description where the nucleus of mass number $A=Z+N$ is considered as a many-body system of A interacting nucleons (Z protons and N neutrons). This should not be confused with an ‘independent particle description’, where a nucleus is described as usually one (or a few) particles moving in an average field created by the rest or by the totality of nucleons in the nucleus and described by semi-empirical parameters. Whereas I personally consider an “individual particle description” as a fundamental approach, I must admit the technical difficulties encountered. For practical purposes I am quite willing to use the semi-empirical approximations. I have, however, a (vain?) hope that some day we may succeed in using the fundamental approach. It is clear that the yrast region is such a border line, because when this region is reached the nucleus spins as fast as possible with a finite number of constituents having oriented angular momenta. But are there other ways of assessing such limits?

What is now the major difference between the results obtained with a genuine many-particle description and with a continuum model? To my mind the major difference is to be found in the number of levels of this or that class which obtain. An example of this

can be found apparently in the number of levels in a rotational band. A few years ago an attempt was made to answer such questions. I feel that it would be interesting to pursue this matter in much greater detail. Results of this kind would also be of interest for the subject of the next question.

Where do the mesonic degrees of freedom come in?

Perhaps this question may be most easily discussed by considering a few historical facts. After the Second World War nuclear physics had become an ‘important science’. Many physicists were optimistic that the ultimate solutions to problems in nuclear physics would soon be found. One such attitude was a widespread hope that bombarding hydrogen with protons and neutrons of energies up to a few thousand million electron volts would provide a complete understanding of nuclear forces. Thus the field of high energy physics flourished with man-made fast projectiles joining cosmic rays as tools.

However the world of minuscule particles is much more complicated than imagined. It turned out that elementary particles appear in large families, that nucleons exist in excited states, that they have structure and internal degrees of freedom.

In the picture of nuclei composed of nucleons these degrees of freedom are neglected. This is a parallel to the fact that when discussing molecules the internal degrees of freedom of the nuclei are usually neglected. My question is really a query into the limit of accuracy to which it is permissible to disregard the mesonic or quark degrees of freedom. Last year the School of Nuclear Physics held at the Ettore Majorana Centre of Scientific Culture at Erice, Sicily, and directed by Denys Wilkinson, was exclusively devoted to this question.

The exotic degree of freedom called strangeness (the characteristic quantum number of kaons and hyperons) has

been clearly identified in nuclear matter, forming the field of study of hyper-nuclear spectroscopy. How soon before we see charming nuclear spectroscopy? Thus there exists some knowledge of the level at which the effects appear. Similarly, excited nucleons (with strangeness zero), the so-called N^* 's, have been considered in nuclear matter in the same way as they are considered in hadron-nucleon collisions, that is, as resonances, but now broadened by Doppler motion. But my question should really be reformulated: when N^* 's, hyperons and so on are produced on nuclei is a nucleus then seen as a collection of quasifree nucleons, or can the effects of the nucleon binding, of the collective of particles forming the nucleus, and of the presence of pions during those exchanges that are responsible for the nuclear forces be discerned? Formulated in this way, it is evident that we ask questions which must be answered by the selection of rare situations or by very high precision. So far, the general feeling is that we have not succeeded in finding the answers. There has been a puzzling result, however.

Collisions between fast nucleons take times T_R of the order of R/c , with R being the nucleon radius in question and c the velocity of light, whereas experiments with fast projectiles take times T_L of the order L/c , where L is the length of our apparatus; the ratio of these time-scales is $T_R/T_L \approx R/L \approx 10^{-14}$ to 10^{-17} . Thus what is seen is something that has been fully developed in the apparatus, but may have very different aspects over periods of time that are but a few times T_R . Here the nuclei may be used as detectors enabling observations over nuclear distances R_N in the range $R \leq R_N \leq 6 \cdot R$ to be made. Developments in ordinary material extending over metre distances can be called 'macroscopic' and developments over nuclear dimensions and with nuclear densities 'microscopic'. The three main results may be summarised as follows (see for example Cronin *et al.* 1976 *Tbilisi Conference on High Energy Physics*).

Macroscopically, cascades of particles produced near the forward direction are found, that is the incoming hadron produces fast particles, which in turn produce fast particles and so on: microscopically, no such cascading results.

If the final state is a 3π or a 5π state, that is three pions produced or five pions produced, then macroscopically the chance of collision (the cross section) is respectively three times and five times that of a single pion; microscopically, the cross section is very closely equal to that for one pion in both cases.

When very large momenta are car-

Variable DNA repeat lengths

from Rosalind Cotter

AFTER the revelation of the chromatin beaded structure there followed much tangled discussion about the amount, if any, of string between the beads, which was based chiefly on the different unit sizes generated by micrococcal nuclease in Oregon and Cambridge. The argument has now moved from the level of technical disagreements involving digestion termination conditions or faulty calibration to a careful comparison of DNA repeat size in different types of chromatin. A correlation with genetic activity is beginning to emerge.

Chambon and coworkers have examined the DNA repeat length of subunits in chromatin from higher eukaryotes (*Proc. natn. Acad. Sci. U.S.A.* **73**, 4382; 1976). Cells of mature tissues have nucleosomes containing 196 base pairs of DNA, genetically dormant cells rather more, and cells from actively dividing tissues rather less. It has been suggested that smaller DNA repeat lengths are found in primitive eukaryotes, the value for yeast being about 165 base pairs (Thomas & Furber *FEBS Lett.* **66**, 274; 1976), for *Aspergillus nidulans* 154 base pairs (Morris *Cell* **8**, 357; 1976), while 170 base pairs are found in nucleosomes of *Neurospora crassa* (Noll *Cell* **8**, 349; 1976).

These and more recent studies (Lohr *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 79; 1977) have shown that, at early times of digestion with micrococcal nuclease, the most resistant portion of the repeating unit always has 140 base pairs. These core particles, formerly known as monomers, do not vary among species or from tissue to tissue (Morris *Cell* **9**, 627; 1976) with respect to size, histone content (two each of H2A, H2B, H3 and H4), and internal cleavage pattern by DNase I. The variations in repeat lengths are therefore attributable to the more nuclease-

sensitive DNA spacer between core particles.

Lohr *et al.* observe a distribution of repeat sizes for yeast and HeLa chromatin, ranging from 140 (that is, no spacer) to 165 base pairs in yeast, to 190 base pairs in HeLa. Chicken erythrocyte oligomers however quickly reach constant sizes during digestion which are compatible with a regular array of core particles and 60 base-pair linkers. The authors speculate on a possible correlation between spacing mode and gene activity, which would be expected to be more complex along chromatin from active cells with sizeable transcribed and non-transcribed regions, and more regular in inactive chromatin in order to meet packaging requirements more efficiently.

The Cambridge group suggest that variations in spacer length may be brought about by histone H1. H1 is highly basic and is thought to interact with phosphates of DNA in the spacer regions. H1 from *Aspergillus* has fewer basic residues than *Neurospora* H1, and can therefore presumably protect only a shorter piece of inter-bead DNA. Morris notes a subunit repeat for chicken erythrocyte chromatin which is longer than that of chicken liver chromatin, and might be attributable to the partial substitution in the former of H1 by the more basic H5. He discusses the interesting possibility that specific recognition sites such as promoters might have altered accessibilities depending on whether they are incorporated into nucleosome cores or spacers; if then nucleosomes are phased with respect to recognition signals, this phasing could be altered by different H1-like proteins inducing changes in linker length. H1 is known to vary during development and cell differentiation and may therefore influence the accessibility of recognition sites in active chromatin.

ried over in the collision (that is, when the struck particle gains a large amount of energy), a relatively rare event has occurred. Naively, it would be expected that the chance of such events occurring in nuclear matter would grow with nuclear mass number as the nuclear cross section, that is roughly proportional to $A^{2/3}$. Experimentally, the result is proportional to $A^{1/3}$.

These three main results need explanation and further study both experimentally and theoretically. It is not known whether they are nuclear or

hadronic effects.

All this does not answer the central question: how many degrees of freedom should we consider in nuclear matter? And when are we to talk about a finite number of constituents and when about continuum? These problems are also linked to the next major point.

Are shock waves observed in nuclear matter?

Certain authors maintain that they have seen such waves but for me the interpretation is not clear. First, I have

no clear picture of what is understood by ordinary sound waves and the velocity of sound in nuclear matter. Bohr and Mottelson (*op. cit.*) point out that in nuclear matter one must assume that one deals with zero sound, that is, vibrations that are close to collision-free. If this is the case, then the velocity of sound is likely to be relatively high and shock waves may need even more violent initiation than could first be imagined. To me, the domain is far from exploited and merits much further study both conceptually, theoretically and experimentally.

In addition to the questions raised above, much work needs to be done on nuclei far from stability, including heavy elements and the possible super-heavies. Exotic atoms also present an interesting field; with antimatter available, they can even be envisaged as $A_1\text{--}A_2$ atoms with strong interaction complications.

All this is, of course, mainly academic. The practical nucleus remains. For the time being however, nuclear applications have become "despised and rejected of men".

□

Acid proteases come of age

from Jordan Tang

In the past 10 years, research into the structure and function of proteases has been successful. The exception has been in the area of the acid proteases which has had the status of a stepchild—mainly because of the absence of structural information. But it now seems that the acid proteases have come of age.

Last November, at the 'Conference on Acid Proteases: Structure, Function, and Biology', held at the University of Oklahoma, three new high-resolution crystal structures of microbial acid proteases were reported. The amino acid sequences of two gastric carboxyl proteases, pepsin and chymosin (B. Foltmann, unpublished data) were completed, and a microbial enzyme, penicillopepsin, is near completion. These results have provided a new framework for the study of the catalytic mechanism and the evolution of this group of enzymes. (The proceedings of the Conference will be published as a book in the autumn of 1977).

Among this group, the best combination of X-ray and sequence data is in the acid protease from *Penicillium janthinellum*: penicillopepsin. This collaborative work of two Canadian groups is reported in this issue of *Nature* (Hsu *et al.* page 140). The enzyme is bilobal and has a pronounced cleft between the two lobes. The 2.8 Å resolution electron density map was calculated, using eight heavy-atom derivatives. Although the amino acid sequence of this enzyme is not completed, the sequence of 16 fragments can be aligned against the known sequence of porcine pepsin (Tang *et al.*, *Proc. natn. Acad. Sci. U.S.A.* **70**, 3437, 1973). From this alignment, the polypeptide backbone and the side-chains of 326 residues can be traced in the electron density map with little ambiguity. The crystallographic data of acid pro-

teases from two other moulds give essentially the same secondary and tertiary structures. The enzyme from *Rhizopus chinensis* has been solved at 3 Å resolution by D. R. Davies's group at the US National Institutes of Health and the enzyme from *Endothia parasitica* has been solved at the same resolution by T. L. Blundell and coworkers at the University of Sussex. Although little sequence information is available for these two enzymes, the tracings of the polypeptide chains are quite certain. The folding of the chains are the same in all three enzymes and the secondary structures consist of large numbers of β -sheets with only a few isolated helical turns. The apparent binding clefts are also extremely similar. In all three cases, the clefts extend across the surface of the molecules and are sufficient to accommodate the binding of about 8 amino acid residues of the substrate as suggested from the specificity studies of microbial acid proteases and pepsin.

A close relationship of tertiary structures of porcine pepsin and the microbial acid proteases is indicated by sequence homology. As mentioned above, the fragments of penicillopepsin can be aligned against the pepsin sequence. Computer programmed comparison shows 11 highly conserved regions, including the regions near the catalytic aspartyl residues which will be discussed below. The crystal structure of porcine pepsin is still uncertain. The previously reported 2.7 Å resolution structure (Andreeva *et al.* *Dokl. Akad. Nauk U.S.S.R.* **228**, 480; 1976) shows a considerable number of contacts along the molecular boundary; a revision of the molecular boundary, as well as a new interpretation of this map, is underway. However, a comparison of the tertiary structures of pepsin and the acid protease from *R. chinensis* by

Andreeva (unpublished results), using the data of Davies with the calculation of rotation function, indicates a high degree of similarity—confirming the implication of sequence comparison.

There has never been any doubt that the active centres of all the acid proteases are very much alike. Two active centre aspartyl residues, 32 and 215 in pepsin were identified by means of active site directed modifying reagents—an epoxide and a diazo compound. These reagents also inactivate most of the other acid proteases. In the crystal structure of all three microbial acid proteases, these aspartyl residues are found to be close to each other at the centre of the apparent binding cleft. In the case of penicillopepsin, the chains of Asp-32 and Asp-215 are in intimate contact so that, as suggested by Hsu *et al.*, they may share a common proton between the oxygen atoms of the two aspartyl residues. In addition, Ser-35 is hydrogen bonded to the other carboxyl oxygen of Asp-32. These three hydrogen-bonded residues, Ser-35, Asp-32, Asp-215, are reminiscent of the charge relay system of the serine protease. All three residues are also conserved in the known sequences of acid proteases. The hydrogen bondings could easily explain the low pK_a of Asp-32 (~ 2) and the slightly high pK_a of Asp-215 (~ 4.5).

But, despite these new findings, the detailed catalytic mechanism of acid proteases is unlikely to be solved quickly. First, direct evidence on the position and orientation of substrates or products in the crystals, which undoubtedly will be studied soon, has not been obtained. The possible roles of some groups near the catalytic site (for example Thr-216 and Gly-217) are also uncertain. Second, although the suggestion of the catalytic roles of two aspartyl residues can easily be fitted to the crystal structures, this evidence does not exclude any of the previously proposed mechanisms based on the catalysis by two carboxyl groups. For example, the controversy regarding the presence of an enzyme-amide intermediate in the catalysis of acid proteases is still unsettled.

The binding position of pepstatin, a strong inhibitor for all acid proteases ($K=10^{-10}$ M for pepsin), has been determined at low resolution in the crystal structure of the *R. chinensis* enzyme (Subramanian *et al.* *Biochem. biophys. Res. Commun.* **68**, 875; 1975). This hexapeptide binds as an extended chain in the centre of the apparent substrate binding cleft. This finding agrees with the results of kinetic studies, using pepstatin fragments, which suggest that pepstatin is a transition state analogue of peptic hydrolysis (Marciniszyn *et al.* *J. biol.*

Chem. **251**, 7088; 1976).

The mystery of the mechanism by which the active centre is involved in the intramolecular activation of the zymogens of acid proteases seems to have been intensified by the knowledge of the tertiary structures. No zymogen has been found for the microbial acid proteases, but based on sequence comparison the NH_2 -terminal position aligns closely in the linear structure with that of pepsin. Assuming that the NH_2 -terminal chain in pepsin is positioned in its tertiary structure like those chains found in three microbial enzymes, then this polypeptide chain would be extended away from the active centre with the NH_2 -terminus on the other side of the molecule away from the binding cleft. However, kinetic data indicate that the intramolecular activation of pepsinogen is inhibited by pepstatin and 'competitively' inhibited by a protein substrate (Marciniszyn *et al.*, *J. biol. Chem.* **251**, 7095; 1976). This suggests that pepsin's active centre is involved in the intramolecular activation of

pepsinogen. The manner by which this is accomplished in the zymogen molecule is unclear.

The results of the crystallographic and sequence studies undoubtedly will serve as models in the study of a large group of acid proteases in various biological systems. These include chymosin, the lysosomal cathepsin D and E, renin, acid proteases in blood and seminal plasma, and others. It is now clear that the microbial and gastric acid proteases are derived by divergent evolution from a common ancestral gene. Cathepsin D and renin, which are similar in size to pepsin, may fall into the same evolutionary pattern. If so, then the reason that renin is derived from an acid protease in evolution might be to take advantage of the extended binding cleft. The stringent specificity requirement of eight residues in the substrate of renin thus can be accommodated. These structural developments mark the beginning of more fruitful investigation into structure-function relations of the acid proteases. □

presence of divalent cations (Mg^{2+} , Ca^{2+}) DNase II produces 100 bp repeats from chromatin rather than the normal 200 bp repeats. This is interpreted as a cleavage site's becoming available within the nucleosome in certain conditions. The integrity of the nucleosome is not destroyed even though the DNA is cut. Calf thymus chromatin depleted of H1 however, showed no 100 bp repeats which only reappeared when H1 was readded and Ca^{2+} was present, showing that a certain amount of superstructure is necessary for generation of the 100 bp fragments.

The structure of native chromosomes is even more difficult to probe however. A. Worcel (Princeton University) reported the finding of supercoiled loops of DNA of around 400 nucleosomes per loop in genomes of interphase nuclei of cultured *Drosophila* cells isolated in 0.9 M HCl which removes H1. In the electron microscope a very gently prepared whole genome shows a nucleofilament of 100 Å thick with no signs of a beaded structure. Worcel identifies this nucleofilament as the supercoiled thread. DNase I treatment of this preparation appears to partly release the supercoils and produce the beaded string appearance. Worcel suggested that the removal of H1 *in vivo* might be involved in the formation of transcriptionally active chromosome puffs.

R. L. Kornberg (Harvard Medical School) produced evidence that nucleosome formation is random with respect to DNA sequence. If the 140 bp core DNA fragments produced from different copies of the same stretch of chromatin are always the same, single-stranded copies of opposite halves of the set of 140 bp fragments produced by digestion with exonuclease III (which digests DNA from the 3' ends only) should not reassociate. If they carry certain sequences in common, as predicted if nucleosome formation is random with respect to DNA sequence there will be reassociation, and for single-copy DNA sequences from rat liver chromatin, Kornberg's evidence seems to show the latter to be the case. He has also found variable internucleosome lengths in chromatin from the same cell type, results consonant with the finding that nucleosome formation is random.

The use of recombinant DNA techniques has revolutionised the study of gene organisation and sequence. By cloning fragments of the *Drosophila* genome and then mapping the cloned fragments back on to the polytene chromosomes D. Hogness (Stanford University) has identified various families of repeated sequences, some, like the histone genes, tandemly repeated, others, as yet functionally

Eukaryotic chromosome structure

by Eleanor Lawrence

A Royal Society meeting on the Structure of Eukaryotic Chromosomes and Chromatin organised by Professor H. G. Callan and Dr A. Klug, was held in London on 16 and 17 February, 1977.

THE meeting covered various aspects of chromosome structure and function ranging from the fine structure of chromatin to the morphology of transcriptional units to detailed genetic studies at the molecular level.

At the level of chromatin structure, now that the string of beads model is generally accepted, attention is focused on the detailed structure of the nucleosome on the one hand and chromatin superstructure on the other. The role of 'histone' H1 in stabilising chromatin superstructure was brought out by several speakers. Although it does not seem necessary for maintaining the integrity of the nucleosome itself it seems to be essential for maintaining internucleosome connections.

P. Chambon (University of Strasbourg) described experiments which show that although all four histones H3, H4, H2A and H2B are required to assemble a complete nucleosome, H3 and H4 alone with SV40 DNA will produce small beaded structures of 75 ± 10 Å and introduce the charac-

teristic superhelical turns into the DNA.

The physical requirements of DNA replication and transcription have led to the proposal that the nucleosome should perhaps be able to split apart in some way, allowing free movement and replication of the DNA without completely shedding the histone. This feature has been built into current models (see for example Weintraub *et al. Cell* **9**, 409; 1976) and Chambon reported that at very low ionic strength, 'half-nucleosomes' of 90 Å diameter can be seen in the electron microscope.

At low ionic strengths A. J. Varshevsky (Institute of Molecular Biology) has obtained three types of nucleosomal particle—MN1 which appears to be the 'core' particle, MN2 which contains 170 bp DNA and has one H1 molecule associated with each particle and MN3, 200 bp and two H1 molecules per particle. He has also found an array of subnucleosomal particles ranging from 20 bp DNA lengths with no histones attached to particles containing 120 bp and all four histones, but as yet it is not possible to interpret these various subnucleosomal particles in terms of nucleosomal structure.

Biochemical studies on chromatin were reported by H. G. Zachau (University of Munich) who finds that at high salt conditions, or in the

unidentified, dispersed throughout the genome. The histone gene repeat unit contains the five histone genes but unlike those in the sea urchin (reported by M. Birnstiel, University of Zürich), H1, H3 and H2A are transcribed off one strand and H4 and H2B off the other. A repeated sequence is found between H3 and H4 and between H2A and H2B and may possibly contain a bidirectional promoter. Tandemly repeated genes appear to be commonly regulated—all on or all off. Dispersed repeated genes on the other hand are apparently regulated independently and different ones may be turned on in different cell types or at different stages in differentiation. Sequencing of two of the dispersed families shows them to have repeated sequences of 500 bp at either end.

M. Birnstiel described continuing sequence studies on the histone gene repeat unit isolated from the sea urchin *Psammechinus miliaris*. About 25% of the total repeat unit comprising the five histone genes separated by spacer DNA has now been sequenced. The structural gene sequences agree well with those predicted on the basis of amino acid sequence, with minor differences attributable to the source of histone. Parts of the spacers which have been sequenced show no repeated sequences. The *HindIII* spacer at the end of the unit is full of stop codons so is presumably non-coding. Some (presumably non-functional) initiation sequences have also been identified in this region. Spacer regions close to the 5' ends of H1 and H2A show some very non-random conserved base pairs just in front of the Met initiation site. These are presumably ribosome-binding sites but this is not yet certain since they do not resemble the ribosome-binding sites identified on brome mosaic virus.

The next step would be to delete various regions of the unit and see how this affects function. A possible assay system might be that described by John Gurdon (MRC Laboratory of Molecular Biology, Cambridge) in which DNA fragments injected into the nuclei of *Xenopus* oocytes have been shown to be accurately transcribed and in some cases translated.

Transcription of the human histone genes is also being studied in Birnstiel's laboratory. The human repeat unit is found in a stretch of 14.5 kb which if transcribed together would give a polycistronic message of 50S. Such a message has been found in HeLa cells and appears to be processed to a 9S message in the cytoplasm via 17S and 28S molecules. Control of histone gene expression also appears to be post-transcriptional since G2 phase cells (when no new histones appear) still

transcribe messenger.

Another approach to the study of control of gene expression is to look at the organisation of the actively transcribing regions on spread chromatin. C. D. Laird (University of Washington, Seattle) described studies defining transcriptional units on *Drosophila* chromosomes. Analysis of patterns of ribonucleoprotein (RNP) fibre lengths along sparsely-fibred regions of actively transcribing non-ribosomal chromatin suggest specific initiation and termination sites for each transcriptional unit. Discontinuous patterns of RNP fibre length along the transcriptional unit have been seen suggesting perhaps that the RNP fibres have been partially cleaved at some point before transcription is terminated. Contiguous transcription units have been found in embryonic *Drosophila* cells.

The problem of whether the characteristic beaded structure for chromatin is maintained in actively transcribing regions is still not completely resolved, but the weight of evidence now tends to the view that it is not (W. W. Francke, German Cancer Research Centre, Heidelberg; Laird). □

Reactions with excited alpha particles: $^{12}\text{C}(\alpha, \alpha^*)$

from P. E. Hodgson

THERE is now a growing interest in using nuclear reactions with unstable emitted particles in studies of nuclear structure. Among the possible reactions, those leading to the emission of ^8Be are the easiest to use, because the ^8Be decays rapidly into two alpha particles that can be detected in coincidence. Another practicable reaction of this type is the $(\alpha, ^2\text{He})$ reaction, where the unstable ^2He nucleus is detected by the coincidence of the protons into which it decays (*Nature* **264**, 511; 1976). Such reactions open up new possibilities for nuclear spectroscopy, so it is useful to see whether there are any more unstable particles that can be detected in a similar way.

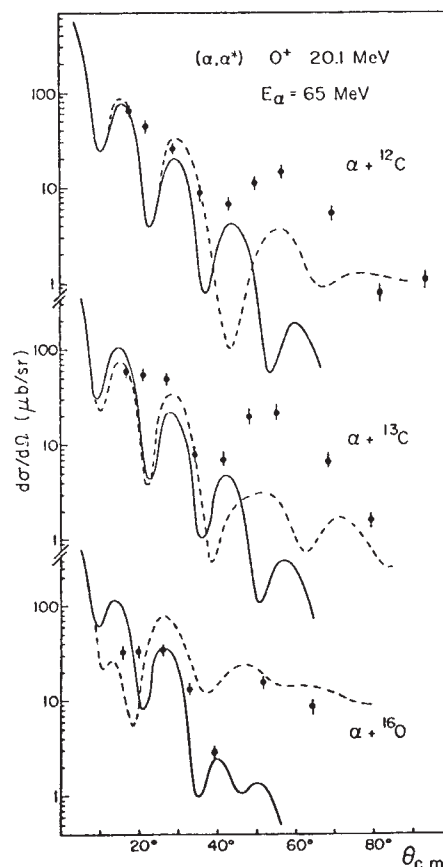
One of the possibilities that has been recently realised is the inelastic scattering of alpha particles with the emission in the excited state at 20.1 MeV. This is an unbound monopole O^+ state with a width of 0.27 MeV. An alpha particle in this state readily decays into a proton and a triton, and since the energy release is only 0.3 MeV the decay products are emitted in almost the same direction and so can easily be detected

by coincidence techniques.

This reaction has recently been observed by Jahn and colleagues (*Phys. Rev. Lett.* **65B**, 339; 1976). They irradiated targets of ^{12}C , ^{13}C and ^{16}O with 65 MeV alpha particles and looked for protons and tritons emitted in coincidence. They found definite evidence that alpha particles are sometimes emitted in the state of monopole excitation, and in the case of the reaction on ^{12}C their energies corresponded to the reactions that leave the residual ^{12}C nucleus in the ground state, in the 2^+ state at 4.44 MeV and in the 3^- state at 9.64 MeV. The cross sections for these reactions are about one-thousandth of those of the corresponding reactions with the emission of alpha particles in their ground state.

Distorted wave calculations were made to see if the observed cross sections could be accounted for by existing theories. The transition matrix elements were calculated microscopically using the particle-hole model and also the collective model. The distorted waves were generated by optical potentials fitted to the elastic scattering of 65 MeV alpha particles.

These calculations agree quite well with the absolute values of the experi-



Differential cross sections for the (α, α^*) reaction to the ground states of ^{12}C , ^{13}C and ^{16}O compared with distorted wave calculations using transition densities obtained from the particle-hole (solid curves) and collective (dashed curves) models.

mental cross sections, but not very well with the details of the angular distributions, as shown in the figure. This discrepancy may indicate the importance of higher order effects, in particular the two-step reactions that proceed through an intermediate state.

The detection of this reaction opens up a new area of nuclear reaction and nuclear structure studies, and in particular suggests that it may be useful to look for the (^3He , α^*) and (d , α^*) reactions as well. $\square$

Tertiary plate motions

from Peter J. Smith

THE relative angular velocities of the Earth's lithospheric plates are now quite well known. The 'absolute' angular velocities of the plates (that is their velocities relative to the mesosphere) are known much less precisely; but if one accepts, with Solomon *et al.* (*Geophys. J.* **42**, 766; 1975), that absolute linear velocities can now be specified to within $1\text{--}2\text{ cm yr}^{-1}$, then it is possible to make three statements, or groups of statements, about their relationships. First, there is a good, but not perfect, general correlation between the velocity of a plate and the fraction of the plate occupied by continent; the greater the proportion of continent the lower is the velocity. At the extremes, predominantly oceanic plates have velocities in the range $7\text{--}10\text{ cm yr}^{-1}$ whereas predominantly continental plates generally move at $0\text{--}2\text{ cm yr}^{-1}$. Second, plates being subducted along a major proportion of their boundaries all have high velocities. And third, lithosphere in the equatorial half of the Earth has a higher r.m.s. velocity than that in the two polar quarters.

These three statements give rise, in turn, to three questions. Do continental plates move more slowly because there is a greater viscous drag at the base of the continental lithosphere than beneath the oceanic lithosphere? Do subducting plates have high velocities because of a major force exerted by the downgoing lithospheric slabs? And does the polar-equatorial velocity differential arise because of a connection between plate motions and the Earth's rotation? It is tempting to allow circumstantial evidence to answer all three questions in the affirmative, or at the very least to argue that the relationships indicated by the three statements have underlying physical

causes. But equally, such relationships could well be coincidental. How, then, can the issue be decided?

The ideal way, as Solomon *et al.* (*J. geophys. Res.* **82**, 203; 1977) point out, would be "to observe a number of global plate systems, each with very different plate geometries but obeying similar physical laws". But that is obviously not possible; so Solomon and his colleagues have done the next best thing in studying the behaviour of the Earth's plates as they were arranged 55 Myr ago. Using the plate boundary map for that period already constructed by Jurdy and Van der Voo (*J. geophys. Res.* **79**, 2945; 1974) together with published values for finite plate rotations, they first determined the relative plate velocities. Absolute velocities were then calculated assuming no net torque to be exerted on the lithosphere for a series of models having driving forces symmetrical about the plate boundaries and a plate-retarding viscous drag at the base of the lithosphere.

Needless to say, such determinations are more complicated than they are made to sound here and involve numerous assumptions, approximations and simplifications that may later give critics cause to make trouble. Taken at face value, however, the results suggest that it may be dangerous to invoke particular physical processes purely on the basis of the behaviour of the plates as they are arranged now. For one thing, with the plates as they were 55 Myr ago there is apparently no correlation between r.m.s. absolute linear velocity and the fraction of the plate comprising continent, assuming the viscous drag to be uniform at the base of both the continental and oceanic lithospheres. Nor is there a clear division between fast-moving predominantly oceanic plates and slow-moving predominantly continental plates. For example, 55 Myr ago the Eurasian plate, 93% of which is continent, had a velocity about equal to that of the Pacific plate. It is true that the Eurasian plate is much slowed down if the uniform drag model is replaced by one in which there is drag only beneath the continental lithosphere; but not only would the latter model violate palaeomagnetic and other constraints, it would actually produce an increase in most continental velocities.

Fifty-five million years ago, plates with major subduction zones were not all moving rapidly either. The Farallon plate, for example, with a large fraction of its boundary under active subduction, was moving at less than half of the rate of the fastest (Kula) plate. On the other hand, the conclusion to be drawn from the polar-equatorial velocity differential is more ambiguous.

The differential undoubtedly existed, albeit in a much weaker form than at present. But whereas the coordinate pole giving the maximum equatorial r.m.s. velocity is now almost coincident with the geographic pole (apparently strengthening the supposition that plate motions and the Earth's rotation are linked), it is 60° from the geographic pole for the older plate distribution. Solomon and his colleagues therefore conclude that the case for a relationship between the Earth's rotation and plate velocities is "not particularly compelling" at 55 Myr and thus, by implication, at present.

On balance, then, this new evidence suggests that the three original questions should be answered negatively; and the fact that affirmative answers are consistent with current plate motions would appear to be a coincidence. But that is not all, for Solomon *et al.* have also been able to bring their analysis to bear on the controversy over whether or not the supposed mantle plumes from which hot spots are derived are fixed in the mantle. By comparing their newly-determined absolute plate motions with observed hot spot traces, they find the fits between azimuths to be variable. For hot spot traces within the Indian and African plates the fits are within expected errors, but there are serious misfits for Pacific plate hot spots and the Icelandic hot spot. In short, not all mantle plumes appear to be fixed with respect to each other and thus not all can be fixed with respect to the mantle. $\square$



A hundred years ago

HIS Majesty, the Emperor of Brazil, observed the eclipse of the moon on the evening of the 27th, at the Arcetri Observatory. The Emperor took a very lively interest in the phenomenon and discussed with acuteness the hypothesis with which Prof. Tempel, the astronomer, and Prof. Echert tried to explain the varying shades and colours in which the moon appeared during the different phases of obscuration. On Monday last his Majesty assisted at a meeting of the Anthropological Society, when Prof. Mantegazza made some interesting remarks on several Maori skulls, and Prof. Giglioli read an elaborate paper on the ethnology of Brazil.

From *Nature* **16**, March 8, 416; 1877.

review article

Binary X-ray pulsars

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There are nine known X-ray pulsars, which are widely believed to be rotating magnetised neutron stars that are accreting matter from their binary stellar companions. The observed pulse profiles provide information on the physics of the accretion process. We present representative profiles of all nine sources for comparison of general features, and we discuss some of these features in terms of the accreting neutron-star model.

THERE are about 150 known galactic X-ray sources. The most widely accepted model, for those sources not associated with supernova remnants, involves a compact object (white dwarf, neutron star, or black hole) in orbit about a relatively normal stellar companion¹. The energy source for X-ray emission by the compact object is derived from the accretion of matter that is supplied by either a stellar wind or critical-lobe overflow from the companion²⁻⁵. During the first systematic X-ray sky survey with the Uhuru satellite⁶, two of these sources were discovered to be regularly pulsing: Centaurus X-3 (ref. 7) (pulse period $P = 4.8$ s) and Hercules X-1 (ref. 8) ($P = 1.2$ s). Evidence for slow pulsation ($P \approx 2$ min) in another source, GX1+4, had previously been obtained from a balloon observation³³. For the X-ray pulsars, it seems very probable that the accreting compact objects are rotating magnetised neutron stars (see, for example, ref. 9 and references therein).

The Uhuru satellite was most sensitive to X-ray pulsations in the period range 0.2–10 s. During the past two years at least six other X-ray pulsars have been discovered. The study of X-ray pulsars has been greatly advanced by the Copernicus, Ariel V, and SAS-3 satellites. Table 1 lists the confirmed X-ray pulsars and, where known, the optical companion. The Crab Nebula pulsar, NP0532, is not included because it is evidently not in a binary system and because its pulsar mechanism is distinctly different from that of the binary X-ray pulsars.

Binary X-ray pulsars as an astrophysical tool

Binary X-ray pulsars provide at least three powerful sources of astrophysical information. First, the rotating neutron star acts as an orbiting clock that enables the observer to measure Doppler effects and thereby determine the size and shape of the orbit about the companion star. This orbital information, coupled with other supplemental data, enables one to place constraints on the masses of both the neutron star and its companion star⁹ and on the size of the companion (see, for example, ref. 48). These quantities are of interest in a wide range of studies from nuclear physics to stellar evolution. Second, information on secular changes in the intrinsic pulse period can help in the understanding of the accretion process near the Alfvén surface (radius $\sim 3 \times 10^8$ cm (refs 2–5)) that surrounds the neutron star. It is at this surface that the dynamics of the accreting matter are first dominated by the intense magnetic field associated with the neutron star and where the spinup or spindown torque on the neutron star is effectively applied. Third, a study of the intensity profiles of the X-ray pulsations may reveal details of the final stages of accretion onto the surface of the neutron star (radius $\sim 10^6$ cm). The

pulse profiles may also be a direct measure of the transmission of the radiation, produced near the surface of the neutron star, through the infalling plasma. Here we discuss recent progress in the study of the pulse profiles of X-ray pulsars.

Pulse profiles

Sample X-ray pulse profiles for the nine binary X-ray pulsars listed in Table 1 are shown in Fig. 1. In all cases, the pulse profiles were obtained by integrating over $\sim 10^2$ to $\sim 10^4$ individual pulses. For several of the pulsars, the pulse profiles do not change significantly with energy over the range 2–10 keV. For others (for example, A0535+26 (ref. 29) and 3U0900–40 (ref. 39)) the dependence on energy is so strong that we display data for one arbitrary narrow energy range only. It now appears that for many of the pulsars, the basic pulse profiles and their energy dependence are preserved over a time scale of at least a year. (The highly variable pulse profiles of GX1+4 seem to be an exception (refs 35 and 67). On the other hand, certain details, such as a strong secondary peak in Her X-1 (Fig. 1), are seen only on occasion.

The profiles in Fig. 1 display a wide range of complexity, ranging from single smooth intensity maxima to five maxima with additional substructure per pulse period. What general features do these profiles exhibit? (1) The profiles are all characterised by large 'duty cycles' that are in the range ~ 30 –100%. This is an order of magnitude larger than those of the narrow pulses observed for radio pulsars⁴⁹. (2) The modulation factors [defined as (maximum intensity minus minimum intensity)/maximum intensity] all fall in the range ~ 0.5 –0.9. (3) At least three of the profiles exhibit sharp features. (We define such a feature as a substantial change in intensity in less than $\sim 5\%$ of a pulse period.) (4) The degree of symmetry of the pulse profiles varies widely from the nearly symmetric shapes of GX301–2 and A1118–61, which contain two times of mirror symmetry per pulse, to the asymmetric shapes of A0535+26 and 3U0900–40 that contain no symmetry times. (5) There is no significant trend in the complexity of the profiles as a function of pulse period. For example, the pulse profiles of SMC X-1 and GX301–2 are somewhat similar in form, as are those of Her X-1 and 3U0900+40. (6) There is a tendency for the profiles of some of the slow pulsars (for example, A0535+26 and 3U0900–40) to vary greatly with energy; at higher energies (not shown in Fig. 1), these pulse profiles become simpler than in the 1–10-keV range.

We note, as have other workers, the apparent existence of a gap in the period distribution of X-ray pulsars, ranging from $P \sim 5$ s to $P \sim 100$ s. As of this writing, the gap seems to

be genuine within the obvious statistical limits and is probably not an observational effect.

Pulsation mechanisms

The most widely accepted mechanism for producing pulsed X-ray emission invokes the channelling of accreted matter to the surface of a rotating neutron star by intense magnetic fields ($\sim 10^{12}$ – 10^{13} G (refs 2–5)). For all of the X-ray pulsars, the pulse profiles are probably determined by the following parameters: the mass accretion rate; the magnetic field configuration near the surface (that is, dipole field or fields of higher complexity); the relative angles between the rotation axis, magnetic axis, accretion disk (if any), and the observer; and the rotation period. Aside from obviously determining the pulse period, the rotation period probably influences the manner in which the accretion across the Alfvén surface occurs⁵⁰.

These considerations suggest two explanations for the production of X-ray pulsations. The first requires a temporal modulation of the accretion of matter onto the surface of the neutron star (ref. 51 and M. Rees, personal communication). This might occur if the accreting material originates in a

In either case, the emergent radiation must then pass through and interact with the infalling magnetised plasma (see, for example, refs 52, 55 and 56). Near the bases of the accretion columns, the magnetic field strengths and particle densities may well be as high as $\sim 10^{13}$ G and $\sim 10^{22}$ cm⁻³, respectively. A variety of photon absorption, emission and scattering processes are possible in these extreme conditions (for example, free-free absorption^{58,59}, cyclotron processes^{50,52,60}, and anisotropic Thomson scattering^{58,61}), all influenced by the strong magnetic field. With the wide ranges of temperature, density, and magnetic field strength that probably occur within the accreting matter, the radiative transfer problem is exceedingly complicated and has heretofore been quantitatively investigated only for specific scattering mechanisms and in various limiting cases of temperature and magnetic field^{52,55,56,62}.

After the radiation has diffused through the infalling medium, it should emerge as X radiation and will have an angular distribution that reflects both the location of the surface hotspots and the radiative transfer through the medium (including possible transfer through matter near the Alfvén surface^{63,64}). This distribution may, in the first approximation and for the

Table 1 Binary X-ray pulsars

Source	Pulse period (s)	Discovery of pulsing	References Data in Fig. 1	Other observations of pulsation	Optical companion
SMC X-1	0.715	10	10	11	Sk 160 (refs 12, 13)
Her X-1	1.24	8	14	15–21, 66	HZ Her (ref. 22)
Cen X-3	4.84	7	23	24–26 and F. Li, unpublished SAS-3 data.	Krzeminski's star ²⁷
A0535 + 26*	104	28	29	30,31	HDE 245770? (ref. 32)
GX1 + 4	122	33†	67	35	M giant star? ^{36,37}
3U0900–40	283	38	39		HD 77581 (ref. 40)
A1118–61*	405	41	41		{ RS Cen? (ref. 42)
GX301–2	696	34	68	J. H. Swank <i>et al.</i> , preprint	{ Be star? (ref. 43)
					WRA 977 (refs 44, 45)
3U0352 + 30	835	46	J. G. Jernigan, unpublished SAS-3 data		X Per (ref. 47)

* Transient source.

† Confirmation of the presence of pulsation was obtained by White *et al.*³⁴.

stellar wind from the companion star, passes through a shock front near the neutron star, and accretes without the formation of an accretion disk (see, for example, S. L. Shapiro and A. P. Lightman, preprint). The accretion may then take place at a variable rate that depends upon the instantaneous angle between the magnetic axis and the direction of the stellar wind. A temporal modulation of accreted material could also occur if an accretion disk surrounds the neutron star and the rotation axis of the neutron star is not perpendicular to the disk. A non-aligned magnetic axis would then cause the accretion disk to pass through different magnetic latitudes as a function of time⁵¹. In either of these cases the pulse profiles would represent, at least in part, the modulation of the rate of matter reaching the surface. The former case, however, predicts an orbital-phase dependence for the pulse profiles (M. Rees, personal communication) that has not been observed to date (unpublished SAS-3 data). In the latter case, the explanation probably fails at least for Her X-1 (which is widely believed to accrete from a disk), where pulse structure has been observed on a time scale of ~ 20 ms (Fig. 1). Since the free-fall time from the Alfvén surface is ~ 0.2 s, it is doubtful that significantly smaller time structure can result from temporal modulation of the accretion rate.

An alternative and more likely picture^{2–5,52–57} for producing the pulsations requires the funnelling of matter along the magnetic field lines to the surface of the neutron star, where the released gravitational potential energy heats a small area on the surface (~ 1 km²) to extremely high temperatures ($\geq 10^8$ K). The radiation from each of the magnetic polar caps distribution that reflects both the location of the surface hotspots (there may be more than two) might be approximately isotropic or it might be beamed by a cyclotron emission process⁵².

case of a simple dipole field, be a function of magnetic latitude only. For a more complex magnetic-field configuration, the emergent X-ray distribution can depend on magnetic longitude as well as latitude. As the neutron star rotates about its axis (which is presumably not aligned with the magnetic axis), a distant observer will pass over a range of magnetic coordinates and will thereby observe a corresponding X-ray pulse structure. It is immediately evident (Fig. 1) that the complex pulse profiles observed for several sources (for example Her X-1, A0535 + 26, and 3U0900–40) cannot be only the result of scattering and absorption in accretion columns associated with a simple dipole field, which would yield profiles with two times of mirror symmetry per pulse.

Conclusions

Of approximately 150 galactic X-ray sources, nine are known to be binary X-ray pulsars. The list is not likely to expand quickly to include many of the remaining sources. Numerous galactic sources have been searched for regular pulsation over a wide range of possible pulse periods with negative results (limits on pulsed fraction of a few percent; unpublished SAS-3 data of S. Rappaport). However, a significant amount of information on the nature of binary X-ray pulsars has already been developed from studies of the nine pulsars discussed in this article. Further observations will probably reveal some new pulsars and these will improve the quality of the statistical information on the properties of these sources. For example, does a genuine gap exist in the pulse period distribution? Are there any systematic trends in the typical pulse profiles as a function of pulse period?

It is possible that the further study of binary X-ray pulsars will not lead in the foreseeable future to any conclusive understanding of the relevant physics and astrophysics involved.

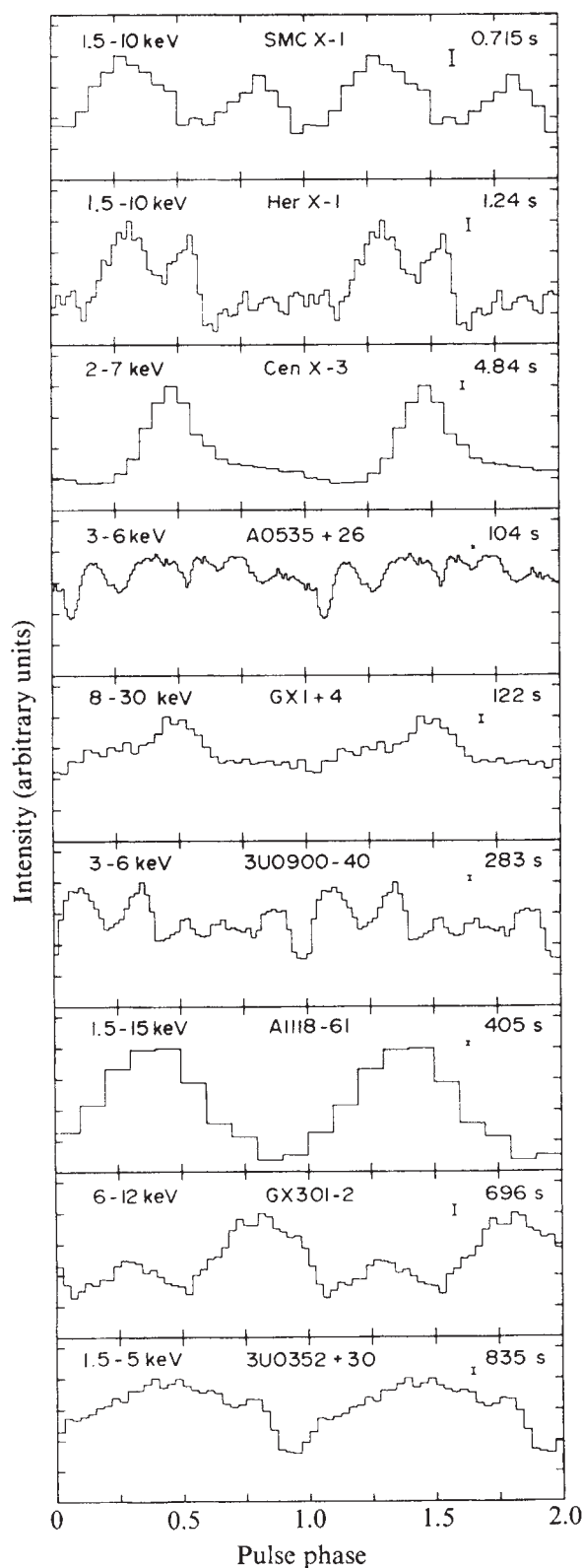


Fig. 1 Pulse profiles for the nine X-ray pulsars that are thought to be in binary systems. In each case, the data (Table 1) are folded modulo the pulse period and plotted against pulse phase for two complete cycles. The approximate pulse periods and energy intervals are indicated for each pulsar. Non-source background counting rates have been subtracted. A typical $\pm 1\sigma$ error bar, derived from photon counting statistics, is indicated for each pulse profile.

This situation may have already emerged in the study of radio pulsars⁶⁵. It might be argued that because binary X-ray pulsars are not isolated objects, their physics may be even more difficult to unravel than that of radio pulsars. It is our belief, however, that while the picture is indeed complex, we already have a sound qualitative idea of the underlying physical processes at work in these systems, and the prospects for further significant advances in our quantitative understanding are therefore encouraging.

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articles

Distances of the Virgo, Fornax and Hydra clusters of galaxies and the local value of the Hubble ratio

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Distances of three nearby clusters of galaxies derived from recent observations of the brightest globular clusters in the brightest galaxies are now in good agreement with other distance indicators and suggest a local value of the Hubble 'constant' $H = 86 \pm 9 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

RECENT observations have renewed interest in the concept of the brightest globular star clusters in galaxies as stochastic distance indicators subject to size-of-sample effects^{1,2}. The thorough study by Hanes³ of the apparent magnitudes of the brightest globular clusters in 12 of the brightest galaxies in the Virgo I cluster facilitates a better determination of the cluster population and luminosity function in relation to the luminosity of the parent galaxy. Smith and Weedman⁴ reported very faint objects, probably globular clusters, at magnitudes $B \approx 23$ to 24 and fainter, around NGC 3311, the brightest galaxy in the Hydra I cluster. Dawe and Dickens⁵ described faint objects, possibly globular clusters, appearing at magnitudes $B \geq 21.0 \pm 0.6$ (m.e.) around several of the brighter galaxies in the Fornax I cluster.

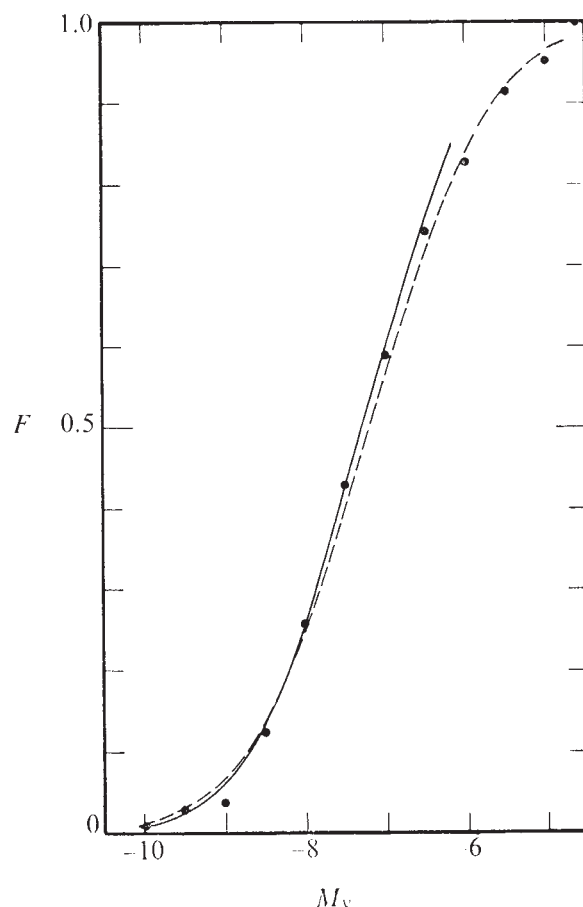
In this paper I first revise the basic parameters, $\bar{M}(\text{CL})$ and $\sigma(M)$, the mean and dispersion of the gaussian model used to estimate the expectation value $M_B(1)$ of the brightest globular cluster as a function of the absolute magnitude $M_B^*(G)$ of the parent galaxy. Second, I introduce a new version of the brightest cluster method which does not require successive approximations. Third, I derive the absolute distance modulus μ_0 of the Virgo I cluster from $M_B(1)$ in the brightest elliptical and lenticular members. Fourth, I derive the distance modulus difference $\delta\mu_0$ between For I and Vir I from several distance indicators, including the suspected globular clusters. Fifth, I derive an approximate modulus for Hya I from the apparent magnitudes and diameters of its brightest members and from $M_B(1)$ in NGC 3311. Finally, I derive the values of the velocity-distance ratio (Hubble 'constant') in the directions of these three nearby clusters, two of which are well outside the boundaries of the Local Supercluster. The result ' $H = 86 \pm 9 \text{ km s}^{-1} \text{ Mpc}^{-1}$ ' is in general agreement with several independent estimates⁶⁻⁹, but in disagreement with others^{10,11}.

Luminosity function of globular clusters and expectation value of $M_B(1)$

The luminosity function of galactic globular clusters is very nearly gaussian (Fig. 1). The cumulative frequency function of the absolute magnitudes listed for 105 clusters by Kukarkin

(ref. 12, Table F) is well approximated by a Laplace-Gauss integral with mean $\bar{M}(\text{CL}) = -7.2$ and $\sigma(M) = 1.2$ mag (dashed curve). The better-defined brighter half, which is of more immediate interest for the evaluation of $M_B(1)$, is more closely fitted by $M_V(\text{CL}) = -7.3$, $\sigma(M < \bar{M}) = 1.1$ mag (solid curve). The latter parameters are adopted in this article. The distance scale used by Kukarkin is defined by the mean magnitude of the RR Lyrae variables in 89 clusters $\bar{M}_V(\text{RR}) = +0.86$

Fig. 1 Cumulative luminosity function $F = N(\text{CL})M_V/N_T$ of 105 galactic globular clusters from Kukarkin's catalogue. The dashed curve is a Laplace-Gauss integral with mean $\bar{M}_V(\text{CL}) = -7.2$ and dispersion $\sigma(M) = 1.2$ mag; the solid curve with $\bar{M}_V(\text{CL}) = -7.3$, $\sigma(M) = 1.1$ mag gives a better fit of the brighter half of the luminosity function.



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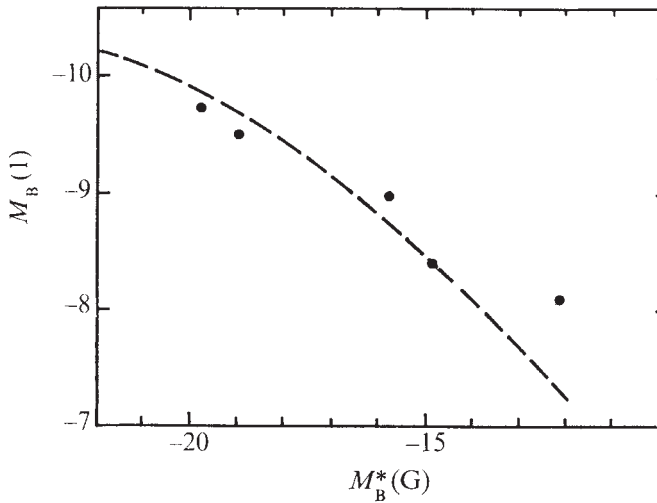


Fig. 2 Observed and computed absolute magnitude $M_B(1)$ of brightest globular cluster as a function of absolute magnitude of spheroidal component of galaxy $M_B^*(G)$. Dashed curve is from statistical theory.

(range 0.57–1.33) (ref. 12 Table 8). A similar fit of the B-band luminosity function gives $\bar{M}_B = -6.55$ and $\sigma(\bar{M}_B < M_B) = 1.1 \pm 0.05$.

The derived expectation value $M_B(1)$ of the brightest cluster depicted in Fig. 2 as a function of $M_B^*(G)$ depends also on the relation between the total cluster population N and $M_B^*(G)$. The adopted relation, $\log N = -0.3 (M_B^*(G) + 11.0)$, is a corrected average of those used in refs 1 and 2. It is rather uncertain and further studies will be necessary to establish this relation more firmly and to determine whether N depends also on other factors. For the present the main justification of the adopted relation is its agreement with empirical data on $B_1(\text{CL})$ for five galaxies of the Local Group covering at least a 5-magnitude range in $M_B^*(G)$ (Table 1). Except for the Fornax dwarf where statistical fluctuations may be expected because of the very small sample ($N = 5$), the O–C residuals are all within ± 0.25 mag which is well within the errors of the data and approximations of the model.

$M_B(1) - M_B^*(G) = B_1(\text{CL}) - B_T^*(G)$ as distance indicator

Derivation of the distance modulus from Fig. 2 requires in general several successive (but rapidly converging) approximations as explained in ref. 1. The need for successive approximations is eliminated in a variant of the method using the difference of apparent magnitudes between the brightest cluster $B_1(\text{CL})$ and the galaxy $B_T^*(G)$ to calculate directly the absolute magnitude $M_B^*(G)$ and apparent distance modulus $\mu = B_T^*(G) - M_B^*(G)$ of the galaxy. Figure 3 shows the relationship between $M_B^*(G)$ and $M_B(1) - M_B^*(G) = B_1(\text{CL}) - B_T^*(G)$ derived from the statistical model and the data in Table 1.

A convenient linear approximation of the relationship for non-dwarf galaxies ($M_B^* \leq -16.0$) is

$$M_B^*(G) = -16.0 - 1.20 (\Delta m_1 - 7) \quad (1)$$

where $\Delta m_1 = B_1(\text{CL}) - B_T^*(G) \geq 7$. It is because $\delta M_B(1)/\delta M_B^*(G) = 0.2$ only that $M_B(1)$ was initially believed to be a constant^{13,14}. Equation (1) can be regarded as an empirical fit of the data which applies irrespective of the validity or otherwise of the underlying statistical model.

Distance modulus of the Vir I cluster

Application of equation (1) to the six brightest E galaxies in the cluster, including M87, with $\langle B_T^*(G) \rangle = 9.9$ and $\langle B(\text{CL}) \rangle = 20.3$, that is, $\Delta m_1 = 10.4$, leads to $M_B^*(G) = -20.1$, and $\mu_B = 30.0$ (the brightest cluster magnitude $B_1(\text{CL})$ is defined statistically by a gaussian fit of the luminosity function¹⁶; it does not depend on the highly uncertain identification of any particularly cluster as the brightest. Galaxy magnitudes

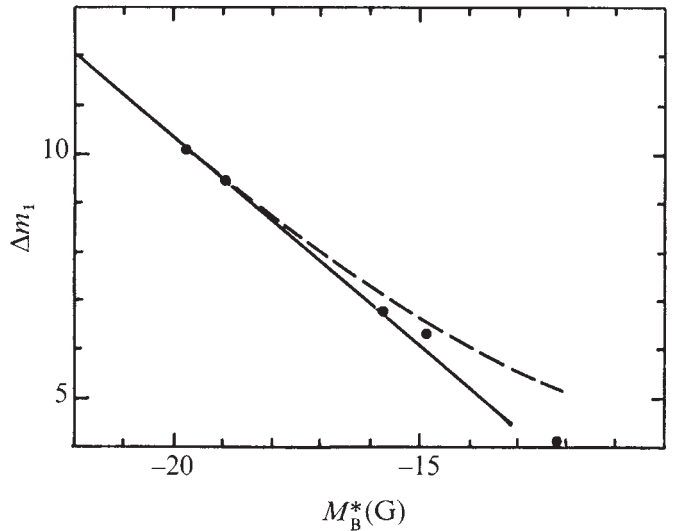


Fig. 3 Relation between magnitude difference $\Delta m_1 = B_1(\text{CL}) - B_T^*(G)$ and absolute magnitude of spheroidal component of galaxy $M_B^*(G)$. The dashed curve is from theory ($\sigma = 1.1$ mag) and the linear approximation is from equation (1).

$B_T^*(G)$ are taken from ref. 15). The five fainter E and L galaxies in the Hanes³ study have $\langle B_T^*(G) \rangle = 10.9$ and $\langle B_1(\text{CL}) \rangle = 20.8$, then $\Delta m_1 = 9.9$, $M_B^*(G) = -19.5$, and $\mu_B = 30.4$. This value is preferred because it does not require an extrapolation of equation (1) beyond the range of $M_B^*(G)$ in Table 1. The mean error of the distance modulus is estimated at 0.3 mag, arising from the following components: 0.1 on $B_1(\text{CL})$, 0.1 on $\bar{M}_B(\text{CL})$, 0.15 on the median magnitude of cluster variables, 0.15 on the basic data¹, and 0.15 from the statistical model and related approximations, including neglect of cluster depth. The adopted modulus of Vir I from the brightest globular clusters is, then, $\mu = 30.4 \pm 0.3$; it is compared in Table 2 with estimates by other methods. The disagreement between the brightest cluster method and other methods⁶ has been greatly

Table 1 Observed and calculated absolute magnitudes of brightest globular clusters

Galaxy	$M_B^*(G)$	$M_B(1)$ (Observed)	(O–C) $\sigma = 1.1$	Δm_1 CL–G	$M_B^*(G)$ (from equation (1))
M31*	–19.8	–9.75	+0.2	10.05	–19.6 _s
Galaxy*	–19.0:	–9.5	+0.2	9.5:	–19.0:
N205	–15.8	–9.0	–0.25	6.8	–15.7 _s
N185†	–14.9:	–8.4	0.0	6.4:	(–15.3:)
Fornax	–12.2	–8.1	–0.7:	4.1	(–12.5:)

* $M_B^*(G)$ for spheroidal component only^{1,6,21}.

† $B_T^*(G) = 10.1$ (ref. 15), $\mu = 24.9$, and $B_{1E} = 16.5 \pm 0.5$ from uncertain $V_1 = 16.2 \pm 0.45$ (ref. 22) and $B_{2,3} = 17.45 \pm 0.05$ (ref. 22) corrected for average $\Delta m(2-1) = 1.15 \pm 0.25$ between second and first ranked clusters in N205 and Fornax.

Table 2 Apparent distance modulus of the Virgo I cluster

Source	Method	$\mu \pm m.e.$
Holmberg ²³	Corrected colour-surface brightness of spirals	30.76
Heidmann <i>et al.</i> ⁷ (Table XXVIII)	Diameter-luminosity relation for spirals in S-cloud	(30.9 $\pm$ 0.5:)
de Vaucouleurs ²⁴ (Table 2, 1965 data)	Group distances from $B(0)$ and $D(0)$, corrected for size of sample	(30.5)
de Vaucouleurs ²⁴	Group distances from $B(0)$ and $D(0)$, corrected for size of sample (E cluster)	30.87 $\pm$ 0.2:
(Table A1, 1973 data)	Group distances from $B(0)$ and $D(0)$, corrected for size of sample (S cloud)	30.51 $\pm$ 0.2:
This article	Brightest globular clusters	30.4 $\pm$ 0.3
	Weighted mean $< \mu >$	30.65 $\pm$ 0.15

Table 3 Distance modulus difference between For I and Vir I clusters

Source	Method	$\delta\mu_0$
de Vaucouleurs ²⁵ (Tables XXI, XXII)	Ratio of mean corrected diameters of E and L galaxies in regions VI (Pavo-Indus) and IIa (For I) divided by ratio of mean $\langle r \rangle$ rings diameters in region VI and Virgo I (0'.85/1'.25)	0.52 (E) (0.12) (L)
de Vaucouleurs ²³ (Table 2, 1965 data, and Table A1, 1973 data)	Group distances from magnitudes and diameters of five brightest and/or largest galaxies corrected for size of sample	(0.8) 0.68 (E,L)
Dawe and Dickens ⁶	$M_1(\text{CL})$ uncorrected for size-of-sample	(0.6) (E)
This article	Corrected magnitudes and diameters of three brightest and/or largest galaxies adjusted for size of sample from RC 2 data ¹⁶	0.97 (E,L)
	Weighted mean	0.7 $\pm$ 0.15

reduced (actually reversed) and all methods now agree within their errors with a mean modulus $\mu = 30.65 \pm 0.15$. Correcting μ for $A_B = 0.20$ mag of galactic extinction, the geometric distance modulus is $\mu_0 = 30.45 \pm 0.15$, corresponding to $\Delta(\text{Vir I}) = 12.3$ (1 ± 0.07) Mpc.

Distance modulus difference $\delta\mu_0$ between For I and Vir I

Several estimates of $\delta\mu_0$ are collected in Table 3; $\delta\mu \simeq \delta\mu_0$ because galactic extinction is very nearly the same in the directions of Vir I ($A_B = 0.20$ mag) and For I ($A_B = 0.21$ mag) in spite of the difference in galactic latitudes¹⁵. Considering the uncertainties of the basic data and underlying assumptions, the agreement between the different methods is rather good; the unweighted mean (rejecting low-weight or preliminary values in parentheses) is $\delta\mu_0 = +0.7 \pm 0.15$, implying a distance ratio $\Delta(\text{For I})/\Delta(\text{Vir I}) = 1.38$ (1 ± 0.07), a geometric distance

Table 4 Distance modulus of Hya I cluster

Method	μ_0
$B_1(\text{CL})$ and equation (1)	33.0
$\Delta B_1(\text{CL})$ from M 31 ($\mu = 24.8$)	33.0
$\Delta\mu_0$ (Vir-Hya) = 2.55 ± 0.2 : from 3 brightest and largest E galaxies	33.0
Adopted mean	33.0 $\pm$ 0.2

modulus $\mu_0 = 31.15 \pm 0.2$ ($\mu = 31.36$), and a distance Δ (For I) = 17.0 (1 ± 0.10) Mpc.

Approximate distance modulus of Hya I cluster

The brightest globular cluster method now agrees with all other reliable distance indicators, so an approximate value of $\Delta(\text{Hya I})$

Table 5 Mean corrected radial velocities of Vir I, For I and Hya I clusters

Source	E, L Types $\bar{V}_0 \pm m.e.$	N	All types $\bar{V}_0 \pm m.e.$	N
a Tammann ¹⁷			1,141 $\pm$ 60	122
b G. and A. de Vaucouleurs ¹⁸	1,012 $\pm$ 71	42	1,054 $\pm$ 66	97
c Sandage and Tammann ^{9,19}			1,111 $\pm$ 75	95
d This article	973 $\pm$ 120	21		
Adopted Vir I	1,000 $\pm$ 70		1,100 $\pm$ 60	
e de Vaucouleurs ²⁴			1,464 $\pm$ 98	12
f Sandage ¹⁰	1,527	4		
g This article	1,462 $\pm$ 108	12	1,448 $\pm$ 89	17
Adopted For I	1,450 $\pm$ 100		1,450 $\pm$ 90	
h Vidal and Peterson ²⁶			3,233 $\pm$ 228	15
i Sandage ¹¹			3,697	7
j This article	3,647 $\pm$ 105	6	3,611 $\pm$ 200	10
Adopted Hya I	3,650 $\pm$ 100		3,500 $\pm$ 200	

a, Galaxies of all types in extended Virgo area, including 'Roberts correction' for spirals. b, 42 Elliptical and lenticular galaxies in 12° E cluster, and 1296 $\pm$ 71 from 26 Sa to Scd galaxies in S cloud; weighted mean is adopted for all types. c, 95 Galaxies of all types in 12° classical cluster (no correction). d, 21 E and L galaxies in 3° = 0.64 Mpc core of E cluster (12h22 to 12h34, +11°. 4 to +14°.3, 1950), mean is 981 $\pm$ 101 ($n = 19$) if 2 objects with large residuals (N4406, N4473) are rejected. e, Galaxies of all types in For I area. f, 4 E and L galaxies in For I area. g, 12 E, L galaxies and 17 of all types in 1°.4 = 0.4 Mpc core of cluster. h, 15 Galaxies of all types in cluster area, includes 10 anonymous objects with $V_0 = 3162$. i, 7 Galaxies of all types in cluster (listed as Hya II), includes 3 anonymous with $\bar{V}_0 = 4280$. j, 6 E, L galaxies and 10 of all types in 0°.5 = 0.35 Mpc core of cluster¹⁶.

Table 6 Velocity-distance ratios for Vir I, For I and Hya I clusters

Cluster	SGL	SGB	V_0 (km s ⁻¹)	Δ (Mpc)	'H'
Vir I (E)	103°	-2°	1,000 (1 ± 0.07)	12.3 (1 ± 0.07)	81 (1 ± 0.10)
Vir I (E+S)	103°	-2°	1,100 (1 ± 0.06)	12.3 (1 ± 0.07)	89 (1 ± 0.10)
For I (E)	262°	-42°	1,450 (1 ± 0.07)	17.0 (1 ± 0.10)	85 (1 ± 0.12)
For I (E+S)	262°	-42°	1,450 (1 ± 0.06)	17.0 (1 ± 0.10)	85 (1 ± 0.12)
Hya I (E)	139°	-38°	3,650 (1 ± 0.06)	40.5 (1 ± 0.1)	91 (1 ± 0.12)
Hya I (E+S)	139°	-38°	3,500 (1 ± 0.03)	40.5 (1 ± 0.1)	87 (1 ± 0.1)
Adopted mean					86 (1 ± 0.10)

may be derived from the work of Smith and Weedman⁴. From their Fig. 2 the apparent magnitude of the brightest clusters in NGC 3311 may be estimated provisionally at $22.8 < B_1(\text{CL}) < 23.8$, say $B_1(\text{CL}) = 23.3 \pm 0.2$. The Galaxy, one of the two brightest ellipticals in Hya I, has a total magnitude $B_T(G) = 12.7 \pm 0.1$ (ref. 15); thus $\Delta m_1 = 10.6$ and, from equation (1), $M_B^*(G) = -20.3$, $M_1(\text{CL}) = -9.7$ and $\mu = 33.0$. Since $M_B^*(G)$ for M 31 and N3311 differ little, a direct comparison of the first-ranked clusters is legitimate; then $\Delta\mu(1) = 23.3 - 15.05 = +8.25$ and $\mu_0(\text{Hya I}) = (24.8 - 0.43) + (8.25 - 0.38) = 33.0$. From a comparison of corrected apparent magnitudes and diameters of the three brightest elliptical members in each cluster, the differential modulus is $\Delta\mu_0(\text{Hya-Vir}) = 2.55 \pm 0.1$: and $\mu_0(\text{Hya}) = 33.0 \pm 0.2$. The adopted mean of the values collected in Table 4 is $\mu_0 = 33.0 \pm 0.2$ and $\Delta(\text{Hya I}) = 40.5 (1 \pm 0.10)$ Mpc (with a galactic extinction $A_B = 0.38$ (ref. 15)).

Local value of the Hubble ratio

Recent or new estimates of the mean corrected redshifts of the Vir I, For I and Hya I clusters are collected in Table 5. To bypass the controversy¹⁷⁻¹⁹ concerning the equality or otherwise of the mean redshifts of the Virgo I E cluster and S cloud, and because For I and Hya I are both E-type clusters with dense cores, the mean redshift of the $3^\circ = 0.6$ Mpc core of the Virgo E-cluster may be considered more appropriate for a direct comparison with the other two clusters. (Also, spirals in the S-cloud have a much larger velocity dispersion^{18,19} and may degrade rather than increase the precision of $\bar{V}_0$.)

The comparison is summarised in Table 6 where the values of the mean velocity-distance ratio ' H ' = $\bar{V}_0/\Delta$ are derived from the adopted redshifts and distances. It is clear that the three clusters are in general agreement with much other evidence to support a mean value ' H ' = $86 (1 \pm 0.1) \text{ km s}^{-1} \text{ Mpc}^{-1}$

as the proper local average of the Hubble ratio, significantly higher than the often quoted values of 57 (ref. 10) to 50 (ref. 11). This persistent disagreement underlines the importance of current efforts to derive the distances of clusters more distant than Vir I in several directions well away from the centre of the Local Supercluster.

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Nile Cone: Late Quaternary stratigraphy and sediment dispersal

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The cyclic nature of Late Quaternary Nile Cone sediments is a response to major climatic oscillations, including marked changes in sea level, Nile River discharge and sediment influx and water mass stratification. Deposits have accumulated most rapidly north-west of the Nile Rosetta Branch since the Early Würm. A broad area of low sedimentation on the Cone, initiated during the Holocene sea-level rise, is predicted to increase in size as a result of the Aswan High Dam and modification of Nile River flow.

THE Nile Cone, largest of the Mediterranean deep-sea fans, has a complex history related to the large-scale tectonics of the Levantine Basin¹⁻³ and evolution of the Nile River which has served as its major sediment source. Deposition on the Cone in the Quaternary has differed from that on most other large subsea fans in one important aspect, that is, sediments carried seaward of the Nile accumulated in a relatively small enclosed water body and thus have been extremely susceptible to climatic changes affecting this region. The deposits record modifications of eustatic sea level, Nile River headwater migration and flow, physical oceanography, including current patterns and stratification,

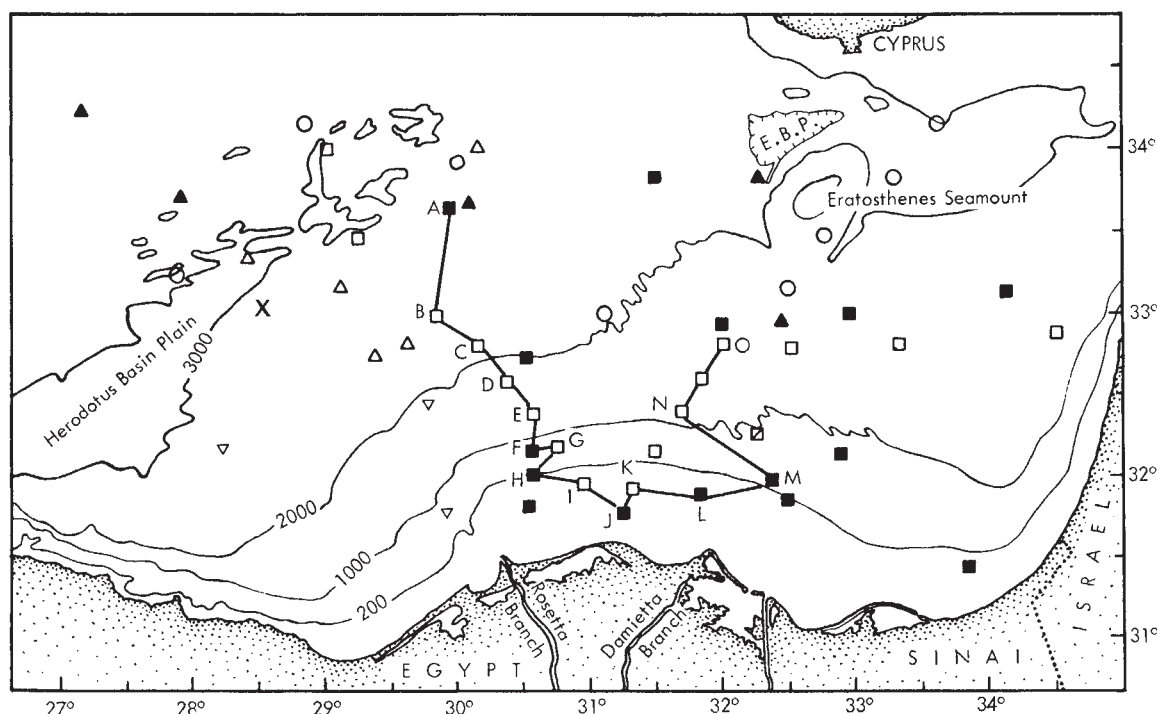


Fig. 1 Chart of the southeastern Mediterranean showing Nile Cone cores examined in this study. EBP, Eratosthenes Basin plain. Depth in metres. ○, Chain 61; ■, Pillsbury 6508; □, Pillsbury 6510; △, Vema 10; ▽, Vema 14; ▲, R. D. Conrad 9; ×, Deep Sea Drilling Project.

and biogenic productivity in front of the Nile. The emplacement of the Aswan High Dam and near-cessation of fluvial input seaward of the delta^{4,5} are now altering the natural evolution of the Egyptian Shelf and Cone. The detailing of Late Pleistocene to Holocene depositional patterns, of which little is known, is of value in predicting changes likely to occur seaward of the subaerial delta.

The stratigraphy of the eastern Mediterranean is discussed in a number of studies⁶⁻¹³. Our earlier investigations emphasised the remarkable variety of sediment types forming the Cone and their cyclic nature^{16,17}. Here, time and spatial variations of the uppermost sedimentary cycles are defined. We believe this cyclicity records large-scale Würm to Recent climatic events which have directly affected the depositional history of the southeastern Mediterranean.

This study entails analysis of 47 piston and gravity cores (Fig. 1), of about 1–15 m length, from the collections of: University of Miami RV Pillsbury cruise 6508 (15 gravity cores) and cruise 6510 (13 gravity and three piston cores); Woods Hole Oceanographic Institution RV Chain cruise 61 (five piston cores); Lamont-Doherty Geological Observatory RV Vema cruise 10 (five piston cores) and cruise 14 (three piston cores), and RV Conrad cruise 9 (three piston cores). Pillsbury and Chain cores are the best preserved. Core analysis includes X radiography, size analysis, total carbonate and organic matter, SEM, clay and sand fraction composition.

A total of 34 Pillsbury core samples provided radiocarbon dates (Table 1, R. Stuckenrath, Smithsonian Radiocarbon Laboratory, personal communication). Dates were obtained from bulk samples about 10–30 cm in length. This method allows a small amount of core section to be used for dating and our tests show that dates so obtained are comparable to those obtained using only the sand-size planktonic foraminifera and pteropod fraction (cf. Pillsbury 6510-14 core section 21–61 cm). X-radiograph analysis reveals that sapropel samples selected for radiocarbon dating are finely laminated and not contaminated by bioturbation.

Correlation shows that sequences in the narrow diameter (4 cm) gravity Pillsbury cores are usually compressed rela-

tive to those in wider diameter (6.5-cm) piston cores, that is, 100 cm of piston core equals about 60 cm of gravity core section (cf. comparative scales along logs in Figs. 2). Piston and gravity cores of the same diameter (6.5 cm) may show a reverse trend, that is, piston core sections compressed relative to gravity core sections (C. Summerhayes, personal communication). Thickness data from piston cores serve as the basis for calculating sedimentation rates; thicknesses in gravity cores are recalculated to correlate with those of piston cores.

Late Quaternary stratigraphy

The simplified graphic logs of 15 of the 47 cores studied in detail are shown in Fig. 2; these are cores for which carbon-14 dates are available. Two well preserved piston cores, Pillsbury 6510-4 (1190 cm long) and 6510-14 (1528 cm long), collected respectively north-east of the Damietta Branch (1634-m depth; Fig. 1, core O) and north-west of the Rosetta Branch (2295 m; Fig. 1, core C) are used as types sections for the chrono- and lithostratigraphical analysis of the Late Quaternary sequence. These cores, collected in two different Cone sectors, show a complete sequence of sediment types without any apparent discontinuity.

The cores on the shelf and upper slope (to about 450-m depth) include three major sediment types in order of decreasing importance: grey homogeneous mud (usually bioturbated), characterised by pelecypods and other benthonic and planktonic components; bioclastic neritic calcareous sand and gravel ('maerl') which display lithological transitions with the grey mud type; and terrigenous shelly sand and silt, including nearshore and pro-deltaic deposits. Two other sediment types, well-represented on the shelf¹⁸ but not usually recovered in cores, are coarse, well-sorted, quartz-rich sands (reworked relict coastal sediment) and calcareous deposits of encrusting algal origin. The upper part of cores on the outer shelf and upper slope usually displays a distinct neritic calcareous sand dated between about 2700 and 2000 yr b.p. The upper part of cores on the inner shelf displays this horizon, and may also show a similar calcareous layer of sub-recent to modern age above it near

the core top (c.f. core Pillsbury 6508-41, Fig. 2). In mid-shelf cores, a lower layer of neritic calcareous sand, separated from the upper calcareous sand by grey mud, is dated at about 16,000 yr b.p. (Pillsbury core 6508-42). The short length of shelf cores precludes an analysis of older Quaternary facies.

Cores collected at depths beyond 550 m on the slope consist essentially of six sediment types: turbiditic sand and silt, turbiditic mud, hemipelagic mud, calcareous ooze, organic ooze and proto-sapropel, and sapropel (defined in ref. 16). Also distinguished is a yellowish-orange to light brown mud termed oxidised layer (OL); where present, it usually caps a sapropel sequence. Clean, well-sorted quartz sands also are present in a few cores on the Cone seaward of Rosetta Branch. In both Pillsbury type cores, hemipelagic

has two distinct calcareous ooze layers at the top separated by hemipelagic mud; it is sometimes difficult to identify this cyclothem, but the well-defined calcareous layer (C_3) of the underlying third cyclothem delineates the base of cyclothem 2. The third (cyclothem 3) shows a well-developed sapropel layer (S_3) but, unlike cyclothem 1, usually includes a reduced proportion of turbiditic layers below the sapropel. This cyclic sequence varies from core to core in terms of thickness and absence of some sediment types.

The age of regionally important stratigraphic horizons in the three cyclothem is determined on the basis of radiocarbon dating (Figs. 2, 3 and Table 1). From top to bottom, the upper calcareous ooze (C_1) is about, or somewhat younger, than 2,700 yr b.p.; the oxidised layer (OL_1) is about

Table 1 Radiocarbon dates based on bulk sample (except P6510-14P, 21–61 cm) from cores selected in different physiographic areas of the Nile Cone and adjacent areas (G, gravity core; P, piston core; p, pilot gravity core)

Core	Type	Depth (cm)	Sediment type	Radiocarbon date (yr b.p.)	Notations
P6508-34	G	0–8	Calcareous ooze	2,795 ± 75	
		100–115	Organic ooze	9,305 ± 85	
P6508-38	G	7–17	Calcareous ooze	2,320 ± 70	
		17–30	Hemipelagic mud	3,265 ± 70	
P6508-38	G	14–25	Biogenic sandy mud	2,705 ± 70	
		45–60	Grey shallow water mud	—	Sample too small
P6508-41	G	24–33	Biogenic sand	2,675 ± 70	
P6508-42	G	88–100	Biogenic muddy sand	16,100 ± 300*	
P6508-45	G	90–110	Grey mud	10,375 ± 290*	
P6510-4	p	25–33	Organic ooze	6,665 ± 295*	
		74–82	Calcareous ooze	17,200 ± 275	
		95–105	Calcareous ooze	19,100 ± 200	
P6510-4	P	11–18	Oxidised layer	5,785 ± 90	
		21–28	Organic ooze	6,575 ± 160*	
		51–60	Sapropel	8,215 ± 160*	
		101–110	Hemipelagic mud	10,475 ± 140*	
		120–129	Hemipelagic mud	15,390 ± 175	
		302–318	Hemipelagic mud	25,875 ± 480	
		421–429	Calcareous ooze/hemipelagic mud	28,725 ± 840	
		461–469	Organic ooze	> 40,000	Apparent age beyond theoretical limits of detection is 45,800 ± 1300
		502–518	Hemipelagic mud/calcareous ooze	> 40,000	
		561–578	Organic ooze	> 40,000*	
		633–648	Hemipelagic mud/calcareous ooze	> 40,000	Apparent age beyond theoretical limits of detection is 46,800 ± 1400
P6510-6	G	40–75	Organic ooze	6,715 ± 200*	
		120–142	Calcareous ooze/hemipelagic mud	16,900 ± 150	
P6510-8	G	102–113	Biogenic sandy mud	—	Sample too small
P6510-10	G	0–10	Biogenic sand	19,700 ± 90	
		90–102	Biogenic muddy sand	—	Sample too small
P6510-11	G	11–20	Calcareous ooze	2,630 ± 70	
P6510-12	G	10–20	Hemipelagic mud	4,350 ± 80	
		80–95	Sapropel	—	Sample too small
P6510-13	G	110–123	Calcareous ooze	17,300 ± 170	
P6510-14	P	21–61	Hemipelagic (turbiditic) mud	5,985 ± 700	Fraction coarser than 63 μ m
		21–61	Hemipelagic (turbiditic) mud	6,325 ± 700	Fraction finer than 63 μ m
		317–360	Hemipelagic (turbiditic) mud	22,500 ± 290	
		531–569	Calcareous ooze/hemipelagic mud	32,600 ± 1000	
		801–839	Sapropel-organic ooze	38,600 ± 700*	
P6510-15	G	100–114	Calcareous ooze/hemipelagic mud	18,830 ± 295	

*Small sample, diluted and counted at reduced pressure.

mud predominates (about 50% of the total core section). The repetition of sediment types which recur from bottom to top is noteworthy: grey hemipelagic mud → sapropel and organic ooze → calcareous ooze. Each complete cycle is defined as a cyclothem¹⁷. Incomplete silt-to-mud turbidite sequences occur indiscriminately throughout each cyclothem (Fig. 2).

The upper three cyclothem present distinctive suites of lithological characters enabling each to be identified in the study area. The uppermost (cyclothem 1) is distinguished by an oxidised layer above a well-marked sapropel (S_1) layer 8–20 cm thick, thick organic ooze layers above and below the sapropel, and a general absence of turbiditic layers above the oxidised layer. The second (cyclothem 2) comprises a poorly developed sapropel (S_2), and usually

5,700 yr b.p.; the upper sapropel (S_1) ranges in age from about 6,600 to more than 8,200 yr b.p.; the calcareous ooze (C_2) top of the second cyclothem is dated about 17,000–19,000 yr b.p.; the second sapropel (S_2) is about 23,000–25,000 yr b.p.; the calcareous ooze (C_3) top of the third cyclothem is dated about 28,000–33,000 yr b.p.; the sapropel (S_3) in this cyclothem is roughly dated at 38,000–>40,000 yr b.p. The top of the fourth cyclothem (C_4) is considerably older than the resolution of carbon-14 method; a maximum age of 55,000–58,000 yr b.p. is estimated based on its position relative to the overlying dated horizons (Fig. 3, log 1).

For calculation of sedimentation rates, the following ages in yr b.p. are assigned to the top of the regionally prevalent stratigraphic horizons: OL_1 = 5,700; C_2 = 17,000; S_2 = 23,000; C_3 = 28,000; S_3 = 38,000; and C_4 = 55,000–58,000. The

sapropel dates correlate relatively well with radiocarbon-dated sapropel sections of some workers¹¹. Our sapropel 2 and 3 dates, however, are much younger than those obtained by other techniques (Ryan¹², for example, attributes an age of about 80,000 yr b.p. to S_3 which is not consistent with our findings).

It is useful to compare the combined litho- and chrono-stratigraphical sequence with generalised eustatic sea-level curves (modified after refs 19–21). The correspondence of cyclothems 1 and 2 with the Bloom *et al.* (1974) data is generally good, while the cyclothem 3 correlation is not so clear cut. The younger, more reliably dated cyclothems 1 and 2 are initiated with slight offset at times of maximum low and high sea levels (Fig. 3). Consideration of the

climatic optimum, at a time when the rate of Holocene sea-level rise had generally decreased. C_2 was deposited before the increase in temperature that resulted in the Holocene rise and probably during a time of slight temperature decrease which may also have been reflected in a slight sea-level lowering. S_2 may correspond to a warmer phase of the Late Wisconsin, and at about the same eustatic position as that of sapropel S_1 , except for generally lower temperatures. C_3 occurs during a cooling phase from the Plum Point interstadial, with perhaps lowering sea level and temperatures. S_3 seems to occur close to the climax of a warmer period. The calcareous top of underlying cyclothem 4 may have accumulated during a cool phase leading into the Port Talbot interstadial (D. J. Colquhoun, personal communica-

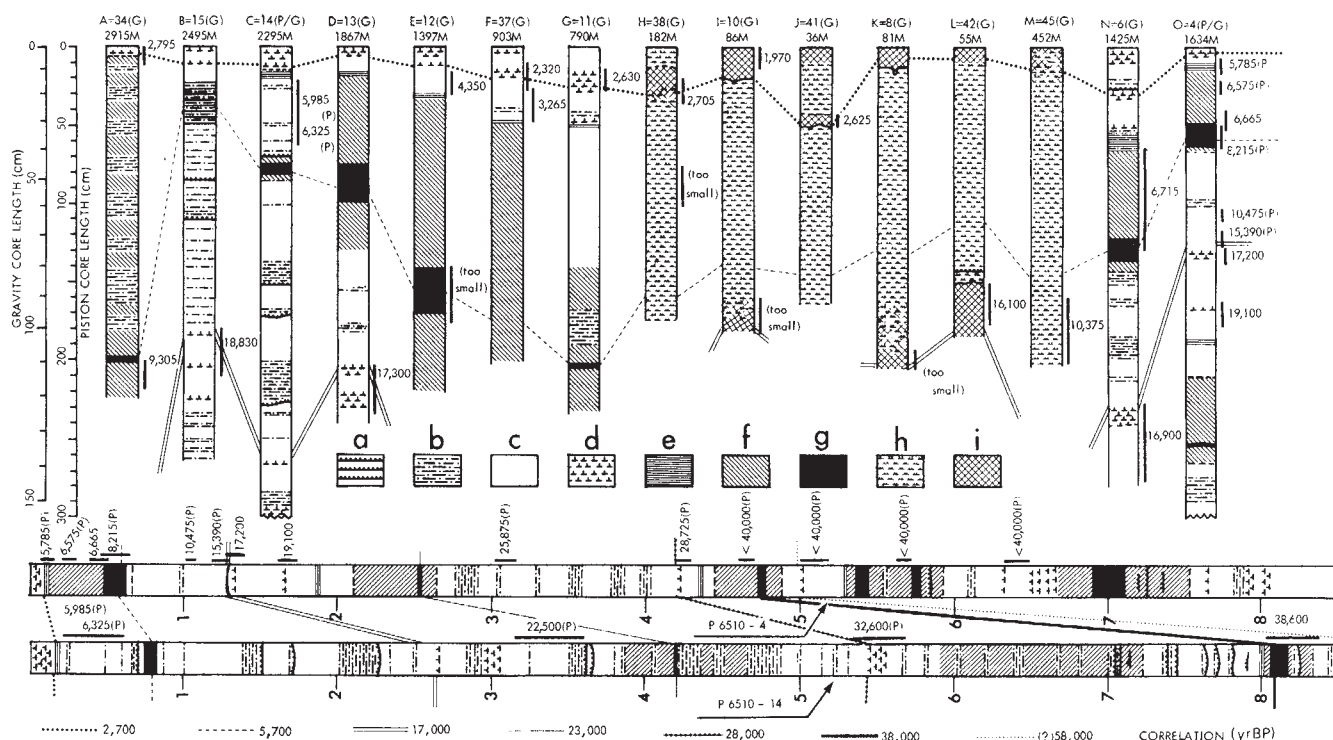


Fig. 2 Correlation of Nile Cone and Egyptian Shelf cores along two transects (position in Fig. 1) based on lithology and radiocarbon dating. Cores P6510-4 and -14 serve as types for defining the Late Quaternary stratigraphy in the southeastern Mediterranean. a, Turbiditic sand and silt; b, turbiditic mud; c, hemipelagic mud; d, calcareous ooze; e, oxidised layer; f, organic ooze and protosapropel; g, sapropel; h, grey (shallow water) mud; i, coarse calcareous sand and gravel. G, gravity core; P, piston core, p, pilot gravity core.

climatic curve based on the Camp Century ice core²² reveals a good correspondence for cyclothem 1 (warming after the Late Wisconsin); cyclothem 2 correlates with the Wisconsin II, between the Plum Point climatic maximum and the beginning of the Holocene rise; cyclothem 3 seems to correspond well with the Middle Wisconsin between about 30,000 and 58,000 yr b.p. including both Plum Point and Port Talbot maxima (D. J. Colquhoun, personal communication).

Calcareous ooze layers, consisting of abundant calcareous nannofossils and Mg-calcite in the form of poorly defined crystals, seem to develop at times of high and low sea-level stands, while the dark organic-rich sapropel layers accumulated during phases of rising as well as lowering sea level (Fig. 3). The relationship between lithology and the Camp Century and eustatic curves suggests the following: C_1 at about 2,700 yr b.p. may have been deposited during a time of slight sea-level lowering and probably cooler (*Littorina*) conditions than earlier. S_1 occurs almost 2,000 yr after climatic amelioration, and generally within the

tion). In this scheme hemipelagic and turbiditic sedimentation should prevail on the Cone at times of maximum sea-level changes when major delta front migration and Nile input alterations would be expected.

Sedimentation rate variations in time and space

The thickness measured between the major dated horizons in cores shown in Fig. 2 serves as a means to calculate sedimentation rates during six time periods in the late Quaternary. Depositional rates are contoured on six charts (Fig. 4) using a geometric scale to emphasise regional variations; the accuracy of contour line positions has been verified by supplemental lithofacies data from 27 Nile Cone cores collected on RV Chain 1975 cruise 119 (D. A. Ross and C. Summerhayes, personal communication). The charts reveal significant changes in sediment dispersal in the study area from the Early Würm to the present.

Low sedimentation rates are recorded during the oldest

phase for which we have reasonable chronological control, that is, from about 58,000 to 38,000 yr BP (C_1 to S_3). The chart depicts minimal rates (about 1–16 cm per 1,000 yr) since it is based on a limited number of cores which are long enough to recover the C_1 layer. The rates may be higher than shown if the age of the C_1 layer is younger than the 55,000–58,000 yr BP postulated. A broad lobe of relatively rapid sedimentation is observed on the upper Cone seaward of both Rosetta and Damietta branches (somewhat higher values occur off the Rosetta Branch). Low values occur elsewhere on the Cone, with lowest values recorded in the vicinity of the Eratosthenes Seamount. Deposits in this earliest time span considered here probably accumulated during several sea-level oscillations (Fig. 3) possibly related to the Port Talbot maximum²². We speculate that the calcareous ooze layer

and 17,000 yr BP (S_2 to C_2). The rates range from 3 to >49 cm per 1,000 yr, and at this time well over half of the area is covered by sediments deposited at >16 cm per 1,000 yr, reflecting the enhanced transfer of material on to the upper Cone sectors. Patterns off the Nile are not distinct; low values occur off the Sinai, and two high sedimentation belts occur on the lower Cone, one extending SSW from Lebanon south of the Eratosthenes Seamount, the other (represented by three isolated areas) between the base of the Cone and the Mediterranean Ridge. The patterns, showing the highest rates over the largest areas, are related to the last major eustatic low stand when prodeltaic deposition extended seaward across the sub-aerially exposed shelf and large amounts of sediment were transported directly on to the slope. Mass gravity as well as hemipelagic and related suspension transport mechanisms

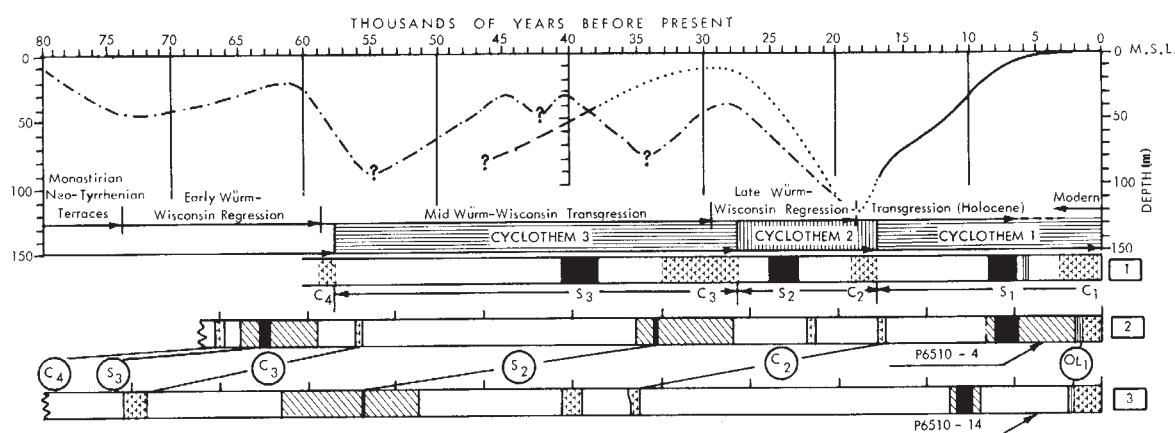


Fig. 3 Correlation between two Nile Cone stratigraphic type cores (logs 2, 3) and a generalised eustatic sea-level curve modified after refs 19–21. Log 1 shows the age of stratigraphic horizons and the upper three cyclothem in thousands of years before present; also shown is the position of the upper three cyclothem relative to sea level. OL₁ — oxidised layer above uppermost sapropel sequence; C_1 , C_2 , C_3 , calcareous ooze layers; S_1 , S_2 , S_3 , sapropel layers; ····· — Curry¹⁹; — — — Fairbridge²⁰; — · — · — Bloom *et al.*²¹.

C_1 (base of cyclothem 3) was deposited during a low eustatic phase (termed the Early Würm²⁰, although no universally accepted sea-level curves are compiled for this period) before an interstadial (Plum Point) at about 30,000 yr suggested by some workers^{19,21}.

The depositional patterns are more distinct during the period between about 38,000 and 28,000 yr (S_3 to C_3). Rates range from about 1 to 26 cm per 1,000 yr, and most of the area is covered by sediment accumulating more slowly than 8 cm per 1,000 yr. A lobe of >16 cm per 1,000 yr is depicted on the Cone seaward of the Rosetta Branch; high values also occur off the Israel-Lebanon margin. This pattern developed either during a general rise in sea-level preceding, and perhaps coinciding with, the interstadial at about 30,000 yr BP (refs 19 and 20), or during a rising-lowering eustatic cycle²¹ (Fig. 3).

Rates between about 28,000 and 23,000 yr BP (C_3 to S_2) range from 1 to >65 cm per 1,000 yr, and are generally higher throughout most of the region than in the previous period, that is almost half of the region is covered by sediments accumulating at >16 cm per 1,000 yr. Two well-marked lobes (>32 cm per 1,000 yr) occur seaward of both branches of the Nile; particularly high values are localised off the Rosetta Branch. This pattern evolved during lowering of sea level and reflects a steady increase of freshwater discharge and sediment supply from the Nile in response to climatic changes affecting the headwaters.

Depositional changes are noted between about 23,000

were involved during this period of marked progradation.

From about 17,000 to 5,700 yr BP (C_2 to OL₁) depositional patterns once again become better defined. Sedimentation rates range from 3 to >88 cm per 1,000 yr; about a third of the Cone surface is covered by sediment accumulating at more than 16 cm per 1,000 yr, and half by 8 cm per 1,000 yr. The highest rates appear channelled on the upper Cone and slope, and a relatively narrow NW-trending lobe extends from the Rosetta Branch towards the eastern part of the Herodotus Basin plain. An east-west zone of low sedimentation (<8 cm per 1,000 yr) forms on the Cone seaward of the Damietta Branch. The more restricted extent of high sedimentation off the delta reflects a progressively decreased input of prodeltaic clastics paralleling the landward shift of the Nile depocentres during the rapid sea-level rise. This last major eustatic shift resulted in the proportionately increased role of hemipelagic sedimentation and diminished terrigenous influx from the Damietta sector of the delta.

Sedimentation rates decrease further during the period between 5,700 yr BP and the present (OL₁ to C_1). Rates range from about 1 to 36 cm per 1,000 yr, and most of the Cone is covered by more slowly accumulating sediments (<8 cm per 1,000 yr). The highest rates occur on the shelf and upper slope, at the foot of the Cone, at the eastern section of the Herodotus Basin plain, and in the Eratosthenes Basin plain. The general decrease of rates away from land and absence of downslope channelisation patterns reflect the primary role of hemipelagic sedimentation and reduction

of turbiditic mud incursions accompanying the stabilisation of sea level at about its present stand. The large east-west belt of low sedimentation is amplified as a result of the diminished role of the deltaic point source and relatively

Mediterranean reflects the interplay of sedimentation with highly variable climatic^{10,26,27}, physical oceanographic²⁸ and biogenic against terrigenous input factors in a quasi-enclosed silled basin. The core data presented here establish a

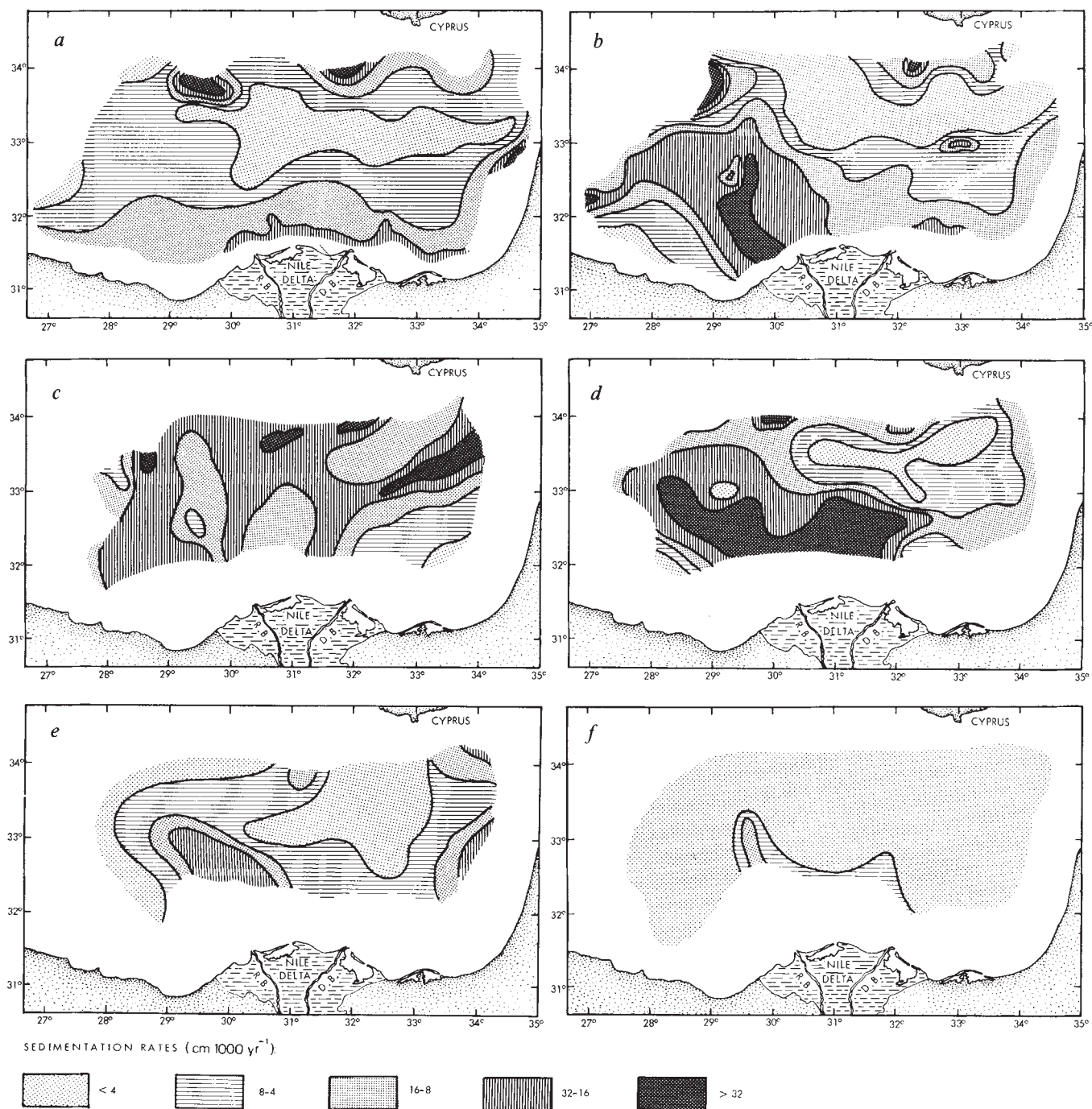


Fig. 4 Charts showing sedimentation rates (in cm per 1,000 yr) on the Nile Cone and adjacent areas during six periods in the Late Quaternary. The stratigraphic horizons used for correlation are shown in Figs 2 and 3. Explanation in text. *a*, OL₁-C₁ (5,700-present); *b*, C₂-OL₁ (17,000-5,700); *c*, S₂-C₂ (23,000-17,000); *d*, C₃-S₂ (28,000-23,000); *e*, S₃-C₃ (38,000-28,000); *f*, C₄-S₃ (58,000-38,000). Bloom *et al.*²¹.

greater importance of lateral suspensate transport and smoothing by predominantly east-west trending surface water flow above the Cone²³⁻²⁵.

Discussion

The orderly and repetitive nature of Late Quaternary sediment types on the Nile Cone and adjacent south-eastern

time-framework for the upper three cyclothem, and provide support for the previously suggested hypothesis that the cyclic nature of major lithofacies in this region is a direct response to climatic factors¹⁷. Specifically, we show that cyclothem correspond closely in time with either major eustatic rise or lowerings of sea level, and that the beginning of each new cyclothem appears slightly

offset (delayed) from the beginning of an oscillation (Fig. 3).

The dated calcareous ooze layers were deposited primarily during cooling temperatures and lowering sea-level stands. Several radiocarbon-dated samples of magnesium-rich layers in other eastern Mediterranean cores (ref. 29, their Table 3) correspond in age to Nile Cone calcareous ooze horizons C₂ and C₃ identified here. Similar calcareous ooze layers of about the same age also are recorded in the western Mediterranean³⁰. The sapropels, on the other hand, accumulated during periods of rising or lowering sea level, and not at times of highest or lowest stands. Earlier work suggested they were deposited primarily during warming trends of climatic curves¹²; our study indicates they accumulated during early stages of temperature maxima. Sapropels record repetitive phases of stratification and near-bottom stagnation^{8, 15, 28, 31, 32}. The general sapropel depositional model is characterised by (1) layering of water masses, (2) stagnation of bottom water with the formation of H₂S-rich zones and (3) seasonal (winter–summer) or periodical changes (eustatic-related upwelling and vertical mixing of water), all of which favour the development and preservation of varve-like bedding^{15, 33}. The oxidised layer found above the sapropels, the third cyclothem lithotype related to climatic and oceanographic phenomena, marks a return to oxygenated bottom-water conditions.

The time and spatial changes in sedimentation rates show the degree to which deposition has been directly affected by the Quaternary climatic–eustatic oscillations. The period of overall highest sedimentation occurred between about 28,000 and 17,000 yr BP; it is during this time span (last major Würm regression) that cyclothem 2 was deposited. As sea level dropped and Nile River discharge patterns altered, an increasing volume of resuspended sediment was transported across large sectors of the delta front, including both the Rosetta and Damietta branches, and moved seaward directly on to the Cone. During this period sedimentation rates over large areas of the Cone are roughly equivalent to the high, long-term average rates (>30 cm per 1000 yr) previously postulated^{3, 14} for this and other submarine fans^{34–37} during the Pleistocene. At times of rising sea level, sediment patterns on the Cone (cyclothem 3 and 1) show more restricted zones of sediment influx localised seaward of the Rosetta Branch, and an extensive east–west belt of low sedimentation over the central and eastern sectors of the Cone. The latter records the diminished input from the delta and the relatively increased importance of suspension processes and regional smoothing by currents responding to large scale water mass flow over the Cone. As might be expected, lowest depositional rates are recorded at times of high sea-level stands, such as exists at present when the influx of the Nile is at a minimum due to the submergence of the Egyptian Shelf, landward regression of the prodelta lobe away from the shelf edge, and reduced freshwater discharge.

The most important zone of accretion on the Cone during the total time span (≈58,000 yr) considered here forms a SE–NW lobe which extends seaward of the Rosetta Branch toward the eastern part of the Herodotus Basin plain. The broad, highly dissected SE–NW trending belt east of this depositional tongue and north of the Damietta Branch¹ does not seem to have had a significant role in downslope channellisation of sediments during the Late Pleistocene–Holocene and even may have served as a barrier to the northward and northeastward transfer of clastics from the delta.

The Late Quaternary cyclothem patterns of the type defined here occurred through much of the earlier Pleistocene, and possibly Pliocene, development of the Nile Cone and, if carefully documented, these older cycles may identify climatically-induced changes back in time. The stratigraphic generalisations can also serve to predict the

future evolution of this submarine fan. In this respect, the emplacement of the Aswan High Dam and cessation of surplus Nile flood water discharging through the delta mouth is bringing to completion a process initiated with the rapid Holocene rise of sea level, that is, the reduction of seaward transported riverborne sediment. The present artificial ‘closing-of-the-faucet’ phenomenon has markedly altered the nature of suspended sediment and nutrients seaward of the delta and reduced the volume of material likely to reach the shelf edge. Immediate predictable results include lowered depositional rates on and beyond the shelf and a broadening of the east–west belt of low accretion on the Cone.

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Monitoring the marine environment through sedimentation

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The argument is advanced that the interception of particles settling within the water column provides a means of monitoring and understanding basic ocean processes. Geochemical and biological measurements on precisely dated bottom sediments and on preliminary recoveries from particle interceptor-traps provide supporting evidence.

ATTEMPTS to monitor and comprehend complex natural systems such as coastal seas and central oceans are characterised by the study of accessible parameters. These parameters are *a priori* presumed to be of importance, and it is further implied that a synthesis of accumulating information will eventually provide a sufficient description of the entire system.

Major problems in scaling arise if the relationship of discrete sampling to the continuous and long-term operation of natural systems is considered. In the case of the ocean this usually translates into the intermittent and non-synoptic measurement of a number of classic factors (such as temperature, salinity, major nutrients, chlorophyll, certain phytoplankton and zooplankton species, nekton and currents). These measurements are made at a number of point locations usually for very short periods, and extrapolations to the seasonal and longer-range character of the ocean are attempted. In such a situation, though there occasionally may be an overwhelming number of samples, there is seldom sufficient information.

An alternative to increasing the number of measurements to gain sufficient data is to enlarge the scale of each measurement, making each observation representative of a relatively large area and significant period of time.

High-resolution bottom sediment records

Study of marine bottom sediments as a record of the overlying ocean is a long-standing and continuing¹ approach to increasing the scale of measurement. The apparent advantage of bottom sediment study is the access to material representative of prehistoric times, and were the open ocean and coastal seas unaffected by human activities, this would be the dominant attraction. If we are concerned with contaminant metals and hydrocarbons in the present marine environment, however, this proposition requires elaboration. The coastal sea lying directly off southern California is an important and convenient region on which to centre discussion.

The four nearshore coastal basins (Santa Barbara, Santa Monica, San Pedro and San Diego) adjacent to the southern California coast fall midway along a broad southward-flowing body of water known as the California Current². The Santa Barbara, Santa Monica and San Pedro basins have impoverished bottom fauna zones as a direct result of their sills intersecting a dissolved oxygen minimum in the water column³, and as a consequence, a virtually

undisturbed sequential record of sedimentary phases accumulates. These basins, particularly Santa Barbara in the north, contain the greatest amount of information to be found in any sediment deposit, and this information can be resolved on time scales approaching 1 yr.

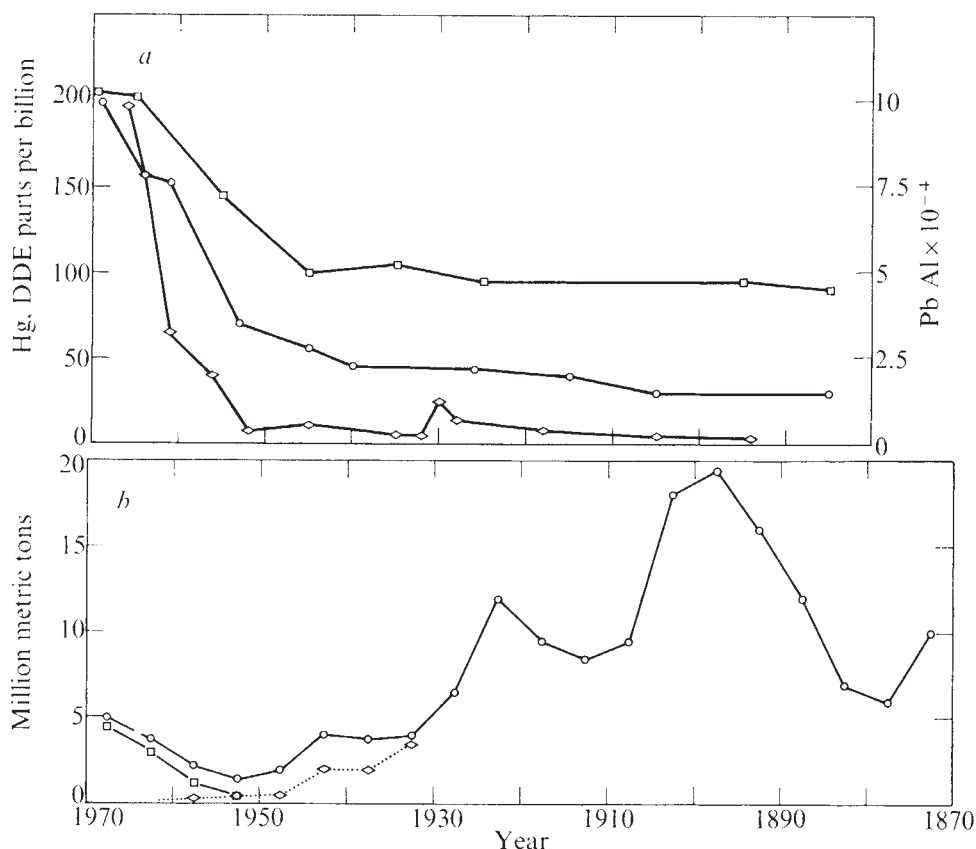
Utilising techniques that include box-coring devices that consistently obtain the exposed sediment surface, verification of surface sediment recovery through the presence of excess ²²⁸Th, and stratigraphic dating by varve and ²¹⁰Pb chronology⁴, detailed historical studies have been performed. In the Santa Barbara Basin sediments, these studies include common lead and its isotopic ratios⁵, mercury⁶ and the halogenated hydrocarbons, DDE and PCB (ref. 7). Metals including Pb, Cr, Cd, Zn, Cu and Ag have been analysed in the surface and near-surface sediments of the three suboxygenated basins⁸. Such studies not only provide a considerable perspective into baseline levels and subsequent anthropogenic increases of certain elements (Fig. 1a) but also most certainly indicate the sensitivity of sediments as an index to changes in the chemistry of the marine environment.

The range of factors available for examination in sediments is not limited to chemical considerations. Formation of a detailed chronological framework in sediments such as those of the Santa Barbara Basin gives a view of the long-term operation of the overlying biological system. This view is limited both by incompleteness of the sediment record and by the investigational effort needed to decipher the record, but is nonetheless remarkable. As an example, it has been found that fish scales of the two historically dominant pelagic species (Pacific sardine and northern anchovy) accumulate in the Santa Barbara sediments, and the accumulation is closely and directly related to fishery-derived population estimates for the entire southern California region¹¹. This relationship leads to an estimate of the variation of fish biomass in past times (Fig. 1b) and also suggests that presently depositing scales are a likely index to present productivity.

A second instance can be drawn from the study of diatom assemblages in these same sediments. If the combined absolute abundances of a number of diatom species, thought to be indicative of warm subtropical waters, are compared through time to an index of California summer surface temperature, it seems that the two records are significantly and directly related¹².

It should be noted that there is internal evidence that information derived from basin sediments applies to a considerable areal scale. Studies of the relationship of regional rainfall patterns to sedimentation (A.S. and P.A.C., in the press) demonstrated that individual laminae in the Santa Barbara sediments often have sufficient morphological character for them to be traced between cores 5 km apart. This is one indication that horizontal forces operating in the water column can effectively disperse and homogenise sediment over large areas; furthermore, in the basin regions off southern California the

Fig. 1 Time series records from dated sediments off California. *a*, Lead^a in the San Pedro Basin (○) and mercury^b and DDE (ref. 7) in the Santa Barbara Basin (□ and ◇ respectively). The salt-corrected dry sediment concentrations of Hg and DDE are given in parts per billion, and Pb as a ratio of Pb/Al $\times 10^{-1}$. Other metal values for the Santa Barbara Basin 1970–71 surface sediments^a are: Mn/Al $\times 10^{-1}$, 51; Fe/Al, 0.63; Ni/Al $\times 10^{-1}$, 7.3; Cr/Al $\times 10^{-1}$, 28; Zn/Al $\times 10^{-1}$, 25; Co/Al $\times 10^{-1}$, 3.4; V/Al $\times 10^{-1}$, 44; Cu/Al $\times 10^{-1}$, 8.3, and Pb/Al $\times 10^{-1}$, 6. Corresponding values for metals in sediment recovered from a particle interceptor-trap deployed 100 m below the surface in Santa Barbara Basin during October 1971 are: Mn/Al $\times 10^{-1}$, 63; Fe/Al, 0.64; Ni/Al $\times 10^{-1}$, 4.5; Cr/Al $\times 10^{-1}$, 22; Zn/Al $\times 10^{-1}$, 107; Co/Al $\times 10^{-1}$, 1.5; V/Al $\times 10^{-1}$, 16; Cu/Al $\times 10^{-1}$, 13; and Pb/Al $\times 10^{-1}$, 16. *b*, Northern anchovy biomass estimates^c (□), Pacific sardine biomass estimates¹⁰ (◇), and combined pelagic fish biomass estimate (northern anchovy, Pacific sardine and Pacific hake) (○) derived from fish scale deposition in Santa Barbara Basin¹¹.



parameter values found in a reliable and documented core are generally good measures of any other reliable core recovered from the same basin, and overall the sedimentation of biogenic and terrestrial debris provide sensitive indices of regional climate, productivity and pollution.

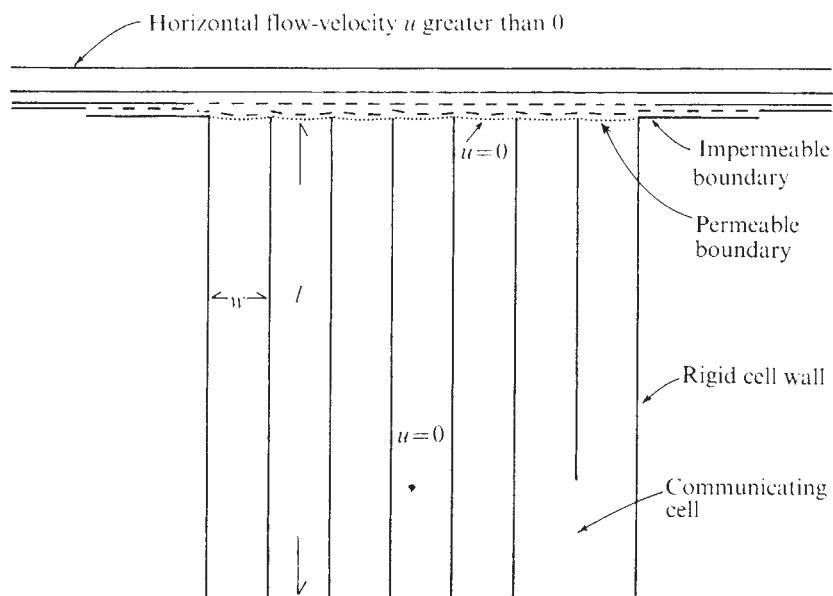
Particulate sediment in the water column

If the bottom of the sea everywhere were similar in character to the Santa Barbara Basin, there would be little need of further elaboration. Varved sediment, however, is quite rare and Santa Barbara is the only known location in the coastal seas off the mainland United States.

Although there are significant occurrences of laminated sediments within the Gulf of California, Mexico and the coastal seas off Peru, South Africa and Pakistan-India, the bottom sediment records that do exist are for the most part thoroughly mixed by benthic organisms and have low to moderate rates of deposition (1–100 cm per 1000 yr). Resolution is thereby limited in such accumulations to time scales greater than 100 yr.

Direct collection of sediment particles in the water column is a means of obtaining information reaching the sediment surface, with potentially higher resolution than most bottom sediment records. Particulates may be directly filtered from the water column by nets¹³ or from water

Fig. 2 Empirical configuration of particle interceptor-traps. Cross-section diagram of closed cell and communicating cell array with horizontal water flow. Provided cells are closed at the bottom (or in the case of communicating cells the length (l) is at least four times the width (w)) and the cell walls are rigid, a quasi-stable boundary regime will be maintained at the cell mouths. Steady horizontal flow (u) will exhibit some drag effects to about $1/5 w$ above the cell mouth; however, depending on the current velocity, penetration of drag-induced vertical movement ceases at $1/5 w$ or less into the cell mouth. For cell widths on the order of 1 cm or less, even with reasonably high current velocities (30 cm s^{-1} highest observed in tank), frictional drag will not induce horizontal currents or vertical turbulence within the cells. In effect the water inside the cells, though still in open diffusional continuity with the overlying flow, is isolated; and only settling particles (and small organisms capable of swimming) may enter and accumulate in the cells. Since the cells are isolated, abiotic agents can be incorporated and maintained as a relatively denser brine at the bottom of the cells to minimise post-depositional grazing and bacterial effects.



samples¹⁴. Such collection provides a measure of particle concentration but suffers from a low yield and the high variability of non-synoptic point sampling. It seems that systematic collection of particulates by filtration would be a useful but limited monitoring strategy.

Interception and collection of sediment in gram amounts and direct assessment of particle flux is possible through the emplacement of particle interceptor-traps within the water column. In conditions of horizontal current flow these devices can, in effect, locally create a hydrodynamic environment¹⁵ similar to that found at the sea floor and

and temporary deposition. Particle interceptor-traps placed near the bottom in such areas can only be used to qualitatively describe sediment in transit. Quantitative collection can ideally be carried out in the waters lying above deeper (> 250 m) basins and gently sloping regions of permanent sedimentation.

Alternatively, the use of particle interceptor-traps is not limited solely to the estimate of particle flux in a long term monitoring context. For example, if concurrent with the interception of particles, the in-the-water particle concentration is measured, then, provided the concentration

Table 1 Dry sediment accumulation in particle interceptor-traps (615 cm²)

Region	Time down (h)	Dry sediment yield (g cm ⁻² yr ⁻¹)	Yield as fraction of long term bottom sedimentation rate*
Santa Barbara Basin 34°14'N; 120°01'W depth 580 m			
May 1969	150 m below surface	50	0.02
	30 m above bottom	50	0.03
December 1969	10 m above bottom	85	0.17
October 1971	100 m below surface	840	0.08
	10 m above bottom	840	0.09
October 1973	150 m below surface	810	0.03
	10 m above bottom	810	0.06
San Pedro Basin 33°30'N; 118°22'W depth 890 m			
May 1972	150 m below surface	640	0.01
	10 m above bottom	640	0.01
Soledad Basin 25°15'N; 112°41'W depth 520 m			
May 1969	150 m below surface	50	0.05
	180 m below surface	50	0.02
	40 m above bottom	50	0.03
	10 m above bottom	50	0.03
October 1973	150 m below surface	590	0.01
	10 m above bottom	590	0.30

*Bottom sedimentation rates²²: Santa Barbara, 0.089 g cm⁻² yr⁻¹; San Pedro, 0.028 g cm⁻² yr⁻¹; Soledad, 0.039 g cm⁻² yr⁻¹.

thereby induce sedimentation. The critical features of a sea-floor boundary region can be produced by a horizontal, open-top/closed-bottom cellular array (Fig. 2).

Sediment trap configurations that have been used in lakes¹⁶ are essentially open-top standing tubes 5–15 cm in diameter. Such configurations, and indeed many others, are well suited for particle collection provided there is little or no horizontal current movement and the particles have a relatively fast settling velocity. In the sea, where near-bottom flow may average 1–5 cm s⁻¹ and on occasion exceed 25 cm s⁻¹, the cellular grid array would seem to be more in harmony with the conditions of sedimentation. The situation can perhaps be visualised if the path of a diatom frustule settling at about 1 m h⁻¹ and being carried along in a 2-cm s⁻¹ horizontal current flow is considered. In this case the tangent path of the diatom will form a vector angle from the horizontal of less than 1°. Furthermore, the grid array configuration facilitates the practical engineering of collecting surfaces of 1 m² or greater necessary to yield sufficient material in the relatively slow sedimentation regime of the ocean¹⁷.

It must be stressed that grid arrays acting as particle interceptor-traps provide a measure of particle flux and, as such, cannot everywhere provide a meaningful measure of sedimentation rates. In nearshore and shallow (less than 100 m) regions there may be considerable resuspension

is reasonably stable, dividing the particle flux ($ML^{-2}T^{-1}$) by the particle concentration (ML^{-3}) gives an estimate settling velocity (LT^{-1}).

Since an initial attempt¹⁸, we have used particle interceptor-traps of 615 cm² for preliminary studies off the coast of California. These traps were routinely deployed on free drop systems¹⁹ consisting of a radio and sight mast, connecting lines of varying lengths, subsurface flotation packs, particle interceptor-traps, timed-release devices and ballast. The system is released from the ship in the stated order and settles to the bottom with traps positioned from 10 m above the bottom to 100 m below the surface. After a preset time, the release closes the traps and releases the ballast or radio mast allowing the system to rise to the surface. In nearshore and other areas of good navigation a less expensive though more vulnerable system can be arranged with a surface marker buoy. Alternatively, an expensive though relatively more efficient arrangement is possible using telemetering acoustic recall devices.

Results that may be useful in considering particle interceptor-traps as a monitoring tool are as follows. The dry sediment yields for a number of drops are given in Table 1. The values for 100–150 m below the surface traps were 0.01–0.08 g cm⁻² yr⁻¹. These projected values range from 0.22 to 1.28 times the underlying long term bottom sedimentation rates. The corresponding values for the

10–40 m above the bottom traps are $0.01\text{--}0.30\text{ g cm}^{-2}\text{ yr}^{-1}$ and $0.33\text{--}7.70$ times the bottom sedimentation rate. One of the extreme bottom values (Santa Barbara, December 1969) has been interpreted in conjunction with hydrographic observations as being due to a density flow deposit (turbidity current)²⁰. The metal composition of sediment recovered from the Santa Barbara, October 1971 trap is given in the legend to Fig. 1a. Although these traps were not especially cleaned for metal investigations, with the exception of zinc, comparison of trap metal values and metal values of surface sediment in the same year are in general agreement.

Major seasonal differences in the aspect of intercepted sediment have been observed. In the Santa Barbara Basin, winter precipitation and storm wave activity lead to distribution and sedimentation of land detritus. Direct transportation of land detritus to the central basin region has been inferred as likely from the collection at 100 m of an essentially clay-silt sediment in the October 1971 trap. This trap was deployed 2 weeks before a substantial

truly used as quantitative instruments in the marine environment.

Monitoring the sea

It is difficult to devise a predictably successful monitoring strategy in the marine environment, for we consistently minimise the intricate nature of the sea and exaggerate our capabilities. Few programmes have ever set out to study persistently and record a substantial part of any marine environment; none have lasted intact much more than a decade.

If we are to assess the effects of future assaults on the seas and oceans, we must begin now to record marine conditions. We must monitor the chemical condition of the sea, paying close attention to the pathways and fate of heavy metals, anthropogenic hydrocarbons and artificial radionuclides. We must monitor the biological condition of the sea, trying to understand or at the least appreciate the fluctuations in productivity and species composition that do occur.

Table 2 Estimated numbers of faecal pellets accumulating in particle interceptor-traps (50-h collection)

Region		Particle size range (μm)				Total
		> 500	500–246	246–124	124–24	
Santa Barbara Basin						
May 1969	150 m below surface	600	6,000	6,000	30,000	42,600
	40 m above bottom	40	2,200	5,000	24,000	31,200
Soledad Basin						
May 1969	150 m below surface	0	50	6,600	~33,000	~40,000
	10 m above bottom	35	20	2,800	~20,000	~23,000

and first rainfall of the season (previous rainfall 6 months before) and was recovered 2 weeks after. Delayed distribution of land-derived detritus has been documented²¹. Sediment initially deposited on the shelf adjacent to the basin is progressively mobilised by subsequent storm waves and disperses towards the central basin on pycnocline surfaces near the 100-m depth. Sediment observed accumulating in traps deployed during the rainy season closely resembles the bulk composition of the Santa Barbara bottom sediments in that it is made up of clay flocculates with abundant silt particles. In contrast to winter deposition, material accumulating in the traps during the spring and summer seasons may on occasion differ substantially in appearance. As an example, in May 1969 in both the Santa Barbara Basin and the Soledad Basin almost the entire fallout was made up of faecal pellets. The pellets contained no recognisable microfossil skeletal fragments in any of the size fractions (Table 2), and the percentage of organic carbon in these samples (18–20%) would indicate a transfer to the bottom of both basins at that time of about $0.2\text{ g carbon m}^{-2}\text{ d}^{-1}$.

In all seasons abundant and representative biogenic debris accumulate in particle interceptor-traps. Fish scales, pelagic molluscs, foraminifera, radiolarians, silicoflagellates, diatoms and coccoliths have all been observed. As a specific example, the group of warm-water indicator diatoms referred to in the bottom sediment discussion were well represented in both the surface and bottom traps deployed in October 1973. It is not possible to relate isolated monthly trap measurements to general temperature information, as little is known about seasonal differences in species abundance and productivity of diatoms off California. But the flux of diatoms in October 1973 projected for the entire year falls within the measured bottom sediment distribution. Furthermore, it should be stressed that all the above results are preliminary and indicate that particle interceptor-traps must be calibrated through year-long studies before they can be

We feel that a unified monitoring of coastal seas and central oceans commensurate with available resources and a common responsibility for the future should soon exist²³. Such an undertaking should involve a number of strategies. We contend that the interception of sediment particulates in conjunction with bottom sediment studies constitutes a major approach.

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Penicillopepsin from *Penicillium janthinellum* crystal structure at 2.8 Å and sequence homology with porcine pepsin

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The polypeptide chain of the acid protease penicillopepsin folds via an 18-stranded mixed β -sheet into two distinct lobes separated by a 30-Å long groove which is the extended substrate binding site. The catalytic residues Asp-32 and Asp-215 are located in this groove and their carboxyl groups are in intimate contact. Alignment of the amino acid sequence with that of pepsin shows regions of high homology.

ACID proteases are important in many diverse biological functions¹. Fungal enzymes have been investigated as potential replacements for chymosin, the acid protease from calf stomach used in cheese production, owing to an ever increasing shortage of rennet. Acid proteases are also used in the preparation of fermented products from rice and soybeans. In mammals acid proteases function not only as extracellular digestive enzymes (pepsin, gastricsin) but also have a role intracellularly in protein turnover (cathepsins D and E (ref. 2)) and in the control of blood pressure (renin, the angiotensin-releasing kidney enzyme³).

Although chymosin has been used as an enzyme solution (rennet) for as long as 6,000 yr and although the first X-ray photographs taken of a protein crystal were those of pig pepsin⁴, the study of the primary and tertiary structures of acid proteases has been slow. The complete amino acid sequence of only pig pepsin⁵ is known and a preliminary communication⁶ on its tertiary structure at 2.7-Å resolution has been published. Low resolution structures of two microbial acid proteases, one from *Rhizopus chinensis*⁷ and the other from *Endothia parasitica*⁸, have also been determined, but no detailed description of the polypeptide chain conformation for acid proteases has been published.

Penicillopepsin, the extracellular acid protease from *Penicillium janthinellum*, is an evolutionary homologue of pig pepsin and chymosin⁹ as judged from a comparison of partial sequences. In addition, its specificity and catalytic mechanism are very similar to those of pig pepsin¹⁰. An aspartic acid residue important in the catalytic mechanism has been identified in a sequence which is nearly identical to one of the active site sequences of pig pepsin^{5,11}.

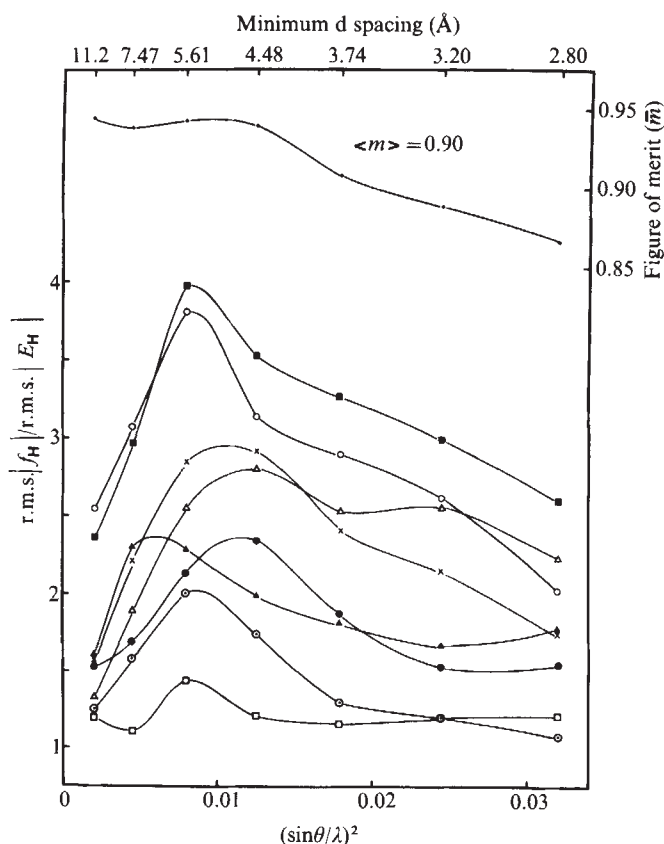
We present here the first high resolution structure of an acid protease in which the complete polypeptide chain can be traced unambiguously. The studies of the structures of the various acid proteases that are currently under way will demonstrate whether or not all these enzymes are homologous and belong to a single enzyme family such as the alkaline serine proteases¹²⁻¹⁶. Similarities and differences in their structures should also help to delineate specific mechanisms and substrate specificities. Our description of

penicillopepsin at 2.8-Å resolution shows features which are likely to be common to many enzymes in this family.

Crystallography

Preliminary data for penicillopepsin crystals (monoclinic, space group C2, cell dimensions, $a = 97.38(8)$, $b = 46.62(3)$, $c = 65.39(6)$ Å, $\beta = 115.4(1)^\circ$) from 2.5 M Li_2SO_4 , 0.1 M sodium acetate, pH 4.4 were reported before¹⁷. A low resolution structure at 6 Å has also been described¹⁸. We used data collection and processing techniques described in the structural studies of the B protease from *Streptomyces*

Fig. 1 Phasing power of the eight heavy-atom derivatives used for the MIR phase determination of penicillopepsin. The ratio of r.m.s. $|f_H|$ to r.m.s. $|E_H|$ and the figure of merit ($\bar{m}$) are plotted as functions of $\sin^2\theta/\lambda^2$. ○, Uranyl acetate; △, $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$; ×, double derivative (Pt : U); ●, mersalyl; □, K_2HgI_4 ; ■, $\text{K}_2\text{UO}_2\text{F}_6$; ▲, phenylmercuric acetate; ⊙, IrBr_3 .



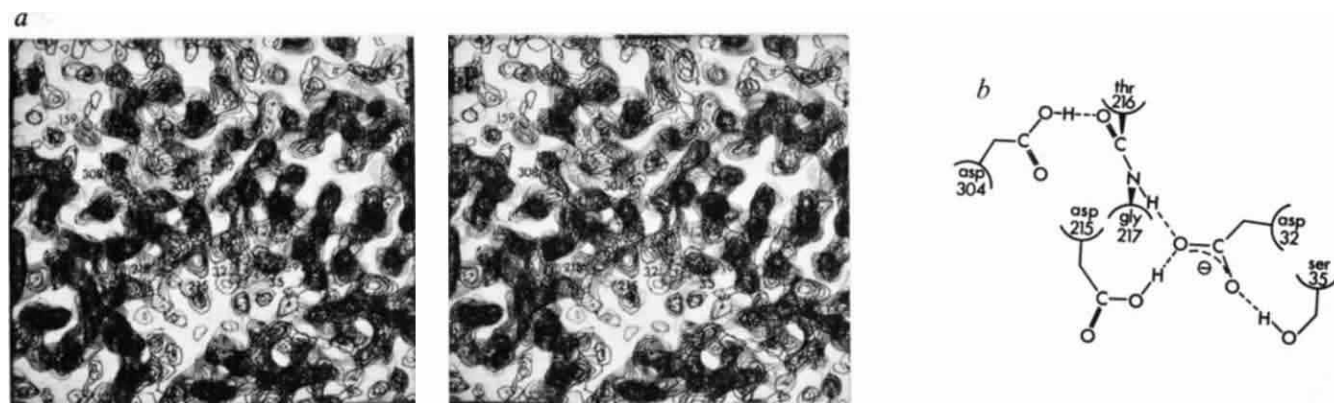


Fig 2 *a*, Stereoview of 10 sections (1-Å spacing) of the electron density map of penicillopepsin in the region of the catalytic site. The sequence numbers (see Fig. 3) are placed close to the side chains in this figure. The close contact of the carboxyl groups of Asp-32 and Asp-215 is in the central portion of the figure and the extended substrate binding groove lies below (in this figure) the active centre region. *b*, A tentative interpretation of the electron density in the vicinity of the active site of penicillopepsin. The carboxyl groups of Asp-32 and Asp-215 are approximately coplanar and share a proton as shown. Asp-32 is involved with two additional hydrogen-bond interactions, one to Ser-35, the other to the main chain NH of Gly-217. Asp-304 is an internally buried side chain and is involved with a hydrogen bonding interaction to the main chain C=O of Thr-216.

griseus^{15,19}. The crystals of penicillopepsin were sufficiently stable to radiation damage in the X-ray beam to allow the collection of the complete 2.8-Å sphere of data (6,700 independent reflections) from a single crystal of the native enzyme and each isomorphous heavy atom derivative. The maximum loss in intensity of the monitor reflections was between 3 and 13% for the various crystals.

Eight heavy-atom derivatives were used to determine the phase angles for the reflections from the native crystals. The heavy-atom binding sites were identified either on difference Patterson maps or cross-phased Fourier maps which made use of the protein phases available from the 6-Å work¹⁸. Refinement of the heavy atom parameters was by the method of least-squares. For two derivatives, K₂HgI₄ and IrBr₃, the final heavy-atom model included two planar trigonal groupings of the HgI₃⁻ ion and IrBr₃. All heavy atom parameters, occupancies, temperature factors and positional parameters, behaved extremely well during the course of the least-squares adjustments with alternating cycles of protein phase angle determination. Table 1 shows the refinement statistics. Figure 1 shows the distribution of the ratio of heavy-atom scattering power (f_H) to lack of closure error (E_H) and of the mean figure of merit ($\bar{m}$) in ranges of $\sin^2\theta/\lambda^2$. The overall mean figure of merit for all observed data to 2.8-Å resolution was 0.90. It is clear from Fig. 1 and Table 1 that all eight heavy-atom derivatives provided useful phasing information to 2.8-Å resolution. The electron density map was computed from the 6,127

(91.4% of total) 'best' phases²⁰ with the measured native protein structure factor amplitudes. The high quality of the map may be judged from Fig. 2 by an examination of several sections computed in the region of the molecule containing the putative active site (Asp-32, Asp-215 in the pig pepsin numbering⁵).

Amino acid sequence

When the 2.8-Å resolution electron density map was computed, the primary structure was available in 16 fragments ranging in length from 3 to 112 amino acids⁹. In addition, there was tentative evidence for four overlaps. The map and the partial sequence data were used in conjunction to place 14 of the fragments in their proper order and to produce the sequence of penicillopepsin shown in Fig. 3. For two regions, 247–264 and 291–302, there was considerable difficulty in correlating sequence data with the electron density distribution. Residues 260–263 are consistent with the sequence of fragment PP6 in ref. 9. Residues 247–259 are consistent with much of fragment PP11 with the exception of the pentapeptide 249–253. In addition, the position of Cys-250 was not confirmed in fragment PP11. We have left residues 249–253 in brackets but the electron density in this region is consistent with the assignment of residues we have made based on the size, shape and spatial surroundings of these side chains. There is no doubt about the assignment of Cys-250 which is involved in the disulphide bridge 250–283. Eight residues from 295–302 satisfactorily account for the electron density in that region of the map and correspond in part to the C-terminal portion of fragment PP12 (ref. 9). Although residue 299 was chemically identified as Phe we have assigned Tyr to this residue based on the electron density associated with the side chain and the fact that a dipeptide Tyr–Leu was obtained in relatively high yield (11%) from a thermolytic digest.

Figure 3 also shows a possible alignment of the penicillopepsin sequence with that of pig pepsin⁵. A final alignment must await comparison to the tertiary structure of pepsin. The sequence information in Fig. 3 was used as input to A. McLachlan's comparison program²¹ which showed 11 well defined regions of high homology (see also Fig. 4). The two regions of highest homology are those in the neighbourhood of the two active site aspartic acid residues (residues 29–42 and 212–221). The relatively high degree of sequence identity (32%) suggests that the tertiary structures of these two enzymes should be very similar with differences in conformation associated mainly with surface loops as has been

Table 1 Heavy atom refinement summary

Derivative	Soaking time (d)	No. of sites*	Range A† (electrons)	Range B‡ (Å ²)	R _c §
1 mM UO ₂ (C ₂ H ₃ O ₂) ₂	7	3	11.5–22.6	6.9–18.4	0.512
5 mM Pt(NH ₃) ₂ Cl ₂	7	2	11.4–43.8	11.4–22.6	0.501
1 mM Pt + 1 mM UA	14	4	8.7–21.2	7.4–17.1	0.581
Mersalyl (saturated)	3	7	5.7–28.4	11.6–25.6	0.587
0.5 mM K ₂ HgI ₄	3	5	5.4–27.6	5.3–20.7	0.626
5 mM K ₃ UO ₂ F ₅	21	4	6.7–31.8	10.9–17.8	0.503
Phenylmercuric acetate (saturated)	7	7	6.6–42.7	9.1–17.9	0.569
2.5 mM IrBr ₃	4	6	4.6–37.4	3.7–43.5	0.653

*Both the K₂HgI₄ and IrBr₃ derivatives had two sites which consisted of trigonal moieties (of HgI₃⁻ and IrBr₃, respectively).

†A is the site occupancy on an approximately absolute scale.

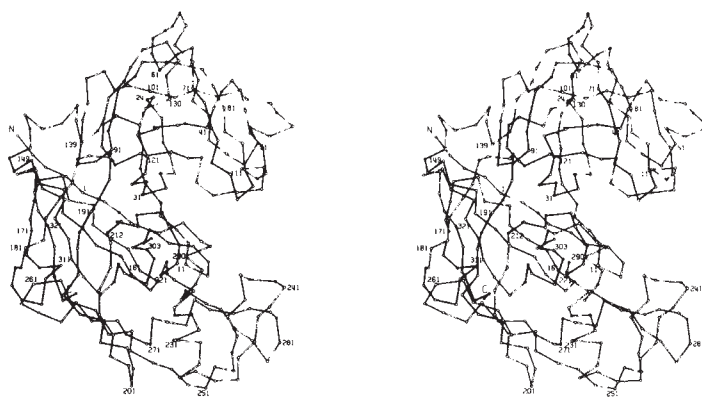
‡B is the isotropic temperature factor coefficient.

§R_c = $\sum ||F_{PH} - F_P| - |f_H|/ \sum |F_{PH} - F_P|$ summed over the centric data only.

	A B																													
	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	15	15	16	17	18	19	20	21	22	23	24
Pen.	A	A	S	G	V	A	T	N	T	P	T	A	N	-	D	E	E	Y	I	T	P	V	T	I	G	-	-	-	-	G
Pep.	A	A	A	L	I	G	D	E	P	L	E	N	Y	L	D	T	E	Y	F	-	-	G	T	I	G	I	G	T	P	A
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Pen.	T	T	L	N	L	N	F	D	T	G	S	A	D	L	W	V	F	S	T	E	L	P	A	S	Q	Q	S	G	H	S
Pep.	Q	D	F	T	V	I	F	D	T	G	S	S	N	L	W	V	P	S	V	Y	C	S	S	L	A	C	S	D	H	N
	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	80	81	82	83
Pen.	V	Y	N	P	S	A	T	G	K	-	E	L	S	G	Y	T	W	S	I	S	Y	G	D	G	S	S	A	S	G	N
Pep.	Q	F	N	P	D	D	S	S	T	F	E	A	T	S	Q	E	L	S	I	T	Y	G	T	G	S	M	-	T	G	I
	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10	11	12	13
Pen.	V	F	T	D	S	V	T	V	G	G	V	T	A	H	G	Q	A	V	Q	A	A	Q	Q	I	S	A	Q	F	Q	Q
Pep.	L	G	Y	D	T	V	Q	V	G	G	I	S	D	T	N	Q	I	F	G	L	S	E	T	E	P	G	S	F	L	Y
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	34	35	36	37	38	39	40	41	42
Pen.	D	T	N	N	D	G	L	L	G	L	A	F	S	S	I	N	T	V	Q	P	Q	S	Q	T	T	F	F	D	T	V
Pep.	Y	A	P	F	D	G	I	L	G	L	A	Y	P	S	I	S	A	S	G	A	T	-	P	V	F	D	N	L	W	D
	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
Pen.	K	S	S	L	A	Q	P	L	F	A	V	A	L	K	H	Q	-	-	Q	P	G	V	Y	D	F	G	F	I	D	S
Pep.	Q	G	L	V	S	Q	D	L	F	S	V	Y	L	S	S	N	D	D	S	G	S	V	V	L	L	G	G	I	D	S
	73	74	75	76	77	78	79	80	81	82	83	84	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	00	01
Pen.	S	K	Y	T	G	S	L	T	Y	T	G	V	D	N	S	Q	G	F	W	S	F	N	V	D	S	Y	T	A	G	S
Pep.	S	Y	Y	T	G	S	L	N	W	V	P	V	-	S	V	E	G	Y	W	Q	I	T	L	D	S	I	T	M	D	G
	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Pen.	Q	S	G	D	G	F	-	-	-	S	G	I	A	D	T	G	T	T	L	L	L	L	B	D	S	V	V	S	Q	Y
Pep.	E	T	I	A	C	S	G	G	C	Q	A	I	V	D	T	G	T	S	L	L	T	G	P	T	S	A	I	A	I	N
	32	33	34	35	36	37	38	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Pen.	Y	S	Q	V	S	G	A	Q	Q	D	S	N	A	G	G	Y	V	F	(T	C	S	B	V)	T	B	L	P	V	S	I
Pep.	I	Q	S	D	I	G	A	-	S	E	N	S	D	G	E	M	V	I	S	C	S	S	I	D	S	L	P	D	I	V
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82	83	84	85	86	87	88	89
Pen.	S	G	Y	-	T	A	T	V	P	G	S	L	I	N	Y	G	P	S	G	N	G	S	T	C	L	G	G	I	Q	S
Pep.	F	T	I	D	G	V	Q	Y	P	L	S	P	S	A	Y	I	L	Q	D	D	D	S	-	C	T	S	G	F	E	G
	90	91	92	93	94	95	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19
Pen.	N	-	-	-	-	S	G	I	G	Y	(L	I	L)	G	D	I	F	L	K	S	Q	Y	V	V	F	D	S	D	G	P
Pep.	M	D	V	P	T	S	S	G	E	L	W	I	L	G	D	V	F	I	R	Q	Y	Y	T	V	F	D	R	A	N	N
	20	21	22	23	24	25	26	27																						
Pen.	Q	L	G	F	A	P	Q	A																						
Pep.	K	V	G	L	A	P	V	A																						

Fig. 3 Comparison of tentative penicillopepsin (Pen) sequence with porcine pepsin (Pep). Amino acid residues enclosed by solid lines are those regions of highest homology when compared with McLachlan's²¹ sequence comparison program. Tentative sequence identity with pig pepsin: 102 residues - 31.7%. The single letter code for the amino acids is given in ref. 46. For penicillopepsin, the order of the fragments from chemical sequencing⁹ with the residue numbers in this table is as follows: N-terminal fragment (-4 to 111); PP3 (111 to 121); PP7 (122 to 125); PP1 (126 to 138); PP2 (139 to 150); central fragment (151 to 197); PP5 (198 to 207); DAN (211 to 220); PP4 (221 to 231); PP10 (232 to 246); PP11 (247 to 259); PP6 (260 to 263); PP12 (265 to 299); X-ray map (300 to 302); C-terminal fragment (303 to 327). Fragments PP8 and PP9 (nine residues) have not been used in this sequence. There is a major discrepancy between the chemical sequence of PP11 and the electron density map. Sequence numbering is that of porcine pepsin⁵. The identification of residues enclosed in parentheses comes mainly from the electron density and not from chemical sequencing and therefore these regions are tentative.

Fig. 4 Stereo-drawing of the main polypeptide chain in penicillopepsin. The circles represent α -carbon positions and only every tenth residue has been numbered (corresponding to the numbering of pig pepsin⁹). The regions of high amino-acid sequence homology (see Fig. 3) are drawn in dark lines. The bilobal feature of the molecule is evident; the active site is located in the groove that separates the two lobes.



observed in comparison of the bacterial serine proteases with the pancreatic enzymes^{15, 16, 22, 23}.

Polypeptide chain conformation

The folding of the polypeptide chain of penicillopepsin is shown diagrammatically in Fig. 4. The overall dimensions of the molecule are approximately $65 \times 49 \times 39 \text{ \AA}$ and this asymmetric shape agrees with the dimensions reported for the low resolution studies of two other acid proteases^{7, 8}. The molecule is distinctly bilobal with an extended hydrophobic interior in the shape of a croissant. The two lobes are separated by a deep cleft which is approximately perpendicular to the $65\text{-\AA}$ molecular length. Buried in the interior of this groove, but accessible to solvent, are the two catalytically important residues Asp-32 and Asp-215. The larger of the two lobes (upper part of Fig. 4) is made up of residues -4 to 171; the smaller lobe is composed of the C-terminal part of the chain.

The largest mixed β -sheet structure observed in globular protein structures is found in penicillopepsin and it consists of 18 strands of polypeptide chain. Figure 5 shows this sheet structure diagrammatically and we have described it according to the convention proposed by Richardson²⁴. Not only is this the largest β -sheet observed in globular proteins but it also contains two cross-overs ($\pm 5X$) that have not been observed previously²⁴. This sheet starts at one end of the molecule (strand e is located in the uppermost portion of Fig. 4) and twists in the usual anti-clockwise fashion by 540° before reaching the final strand o. The central 10 strands are all anti-parallel, and the strands containing the active site aspartic acid residues (strands c and m) both have neighbouring parallel strands which are partly buried in the hydrophobic interior of the molecule. The topology of this sheet produces the croissant-shaped hydrophobic interior and there are 12 'hairpin' turns that are all located on the enzyme's surface.

Those stretches of polypeptide chain which adopt a helical conformation are 58–62, 137–141, 225–235 and 303–309. The single disulphide bridge in the molecule, Cys-250 to Cys-283 is located on the extremity of the smaller lobe (bottom right of Fig. 4). It is curious that this feature which seems to contribute little towards the overall stability of the structure is so highly conserved in acid proteases^{3, 9, 25}. Porcine pepsin has two additional disulphide bridges, 45–50 and 206–210. The corresponding residues of penicillopepsin, although not cysteines, are sufficiently close to be connected by a disulphide bridge.

Catalytic site

Chemical studies of acid proteases, and the pH dependence of the catalytic rate constants of substrate hydrolysis implicate two carboxyl groups in the catalytic event; one

in the protonated form, and the other in the unprotonated form²⁶. The inactivation of porcine pepsin and penicillopepsin by diazoacetylphenylalanine ethyl ester²⁷ and diazoacetyl norleucine ethyl ester¹¹ (DAN) identified Asp-215 (Fig. 3) as one of the catalytic residues. The inactivation of porcine pepsin by 1,2-epoxy-3(*p*-nitro-phenoxy)propane (EPNP) showed that Asp-32 was probably the other catalytic residue²⁸. EPNP also inactivates penicillopepsin²⁹, although it has not been possible to isolate the labelled peptide. We assume that the site of reaction with EPNP is the same as that in pig pepsin because the electron density map (Fig. 2) shows clearly that the two carboxyl groups of Asp-32 and Asp-215 are in intimate contact. In addition, Asp-32 is in a sequence of five amino acids that are identical in the seven acid proteases examined³⁰.

Asp-32 is in strand c (Fig. 5 and legend) which is anti-parallel to neighbouring strand m which contains Asp-215. Both of these strands have neighbouring parallel strands h and p, respectively. Figure 3 shows the high degree of sequence homology for these two pairs of strands and the conformation of the chain in these pairs is very similar; both strand c and strand m have a sharp bend at the two glycine residues 34 and 217. There does not seem to be main chain hydrogen bonding between strands c and m, however there is clearly hydrogen bonding of Thr-33 side chain to the main chain between Ala-214 and Asp-215 and a corresponding interaction of the side chain of Thr-216 with main chain between Phe-31 and Asp-32. Both strands h and p are internal strands, which is usually the case for parallel β structure²⁴.

Maleic acid has two carboxyl groups which interact³¹ and the pK_a values of these groups are 1.83 and 6.07

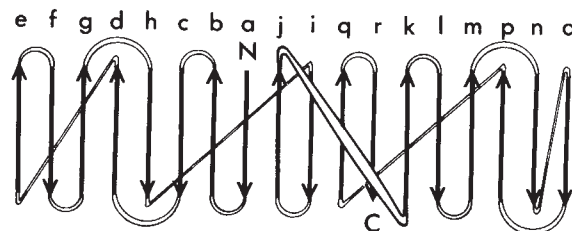


Fig. 5 Diagrammatic representation of the 18-stranded mixed β -pleated sheet structure. This diagram does not display surface topology which can be seen in Fig. 4. Strands of polypeptide chain are labelled a–r from the N to C terminus. The residue numbers at the start and end of each strand are: a ($-2 \rightarrow 8$), b ($11 \rightarrow 18$), c ($25 \rightarrow 33$), d ($36 \rightarrow 41$), e ($62 \rightarrow 66$), f ($84 \rightarrow 87$), g ($98 \rightarrow 104$), h ($118 \rightarrow 124$), i ($150 \rightarrow 156$), j ($161 \rightarrow 168$), k ($179 \rightarrow 186$), l ($189 \rightarrow 199$), m ($202 \rightarrow 216$), n ($218 \rightarrow 224$), o ($286 \rightarrow 295$), p ($298 \rightarrow 303$), q ($309 \rightarrow 316$), r ($319 \rightarrow 325$). The sequence of connection types according to the convention of Richardson²⁴ is $-1, -1, -2, -3X, +1, +2, +5X, -1, +4X, +1, +1, +2, +1X, -2, -5X, +1$.

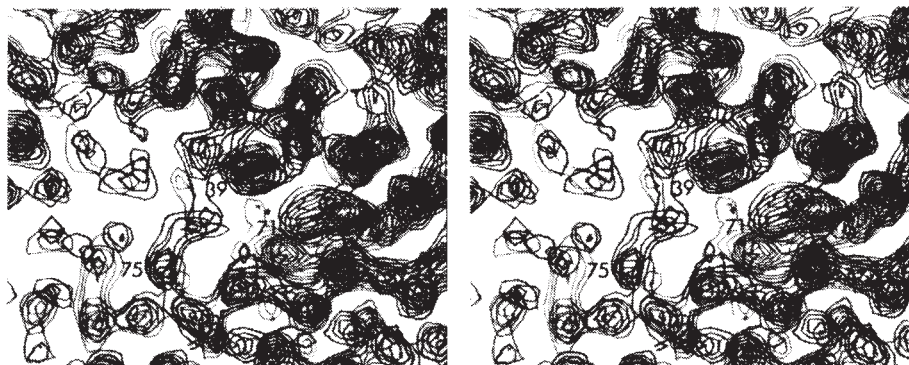
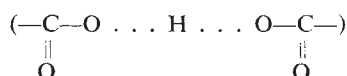


Fig. 6 Seven sections (1-Å spacing) of the electron density map in the region of Trp-39, Trp-71 and Tyr-75. The stacking of the rings of Trp-39 and Trp-71 is shown and the edge of Trp-39 is in contact with the solvent channel of the substrate-binding groove.

(ref. 32). Both maleic acid and the maleate monoanion^{33,34} have a proton shared between two oxygen atoms



The interaction of the carboxyl groups of Asp-32 and Asp-215 probably occurs through a similar sharing of a proton. The proximity of these groups could satisfy the requirement for a protonated and an unprotonated group²⁶. The pH-dependence of the DNA-reaction with Asp-215 (refs 27, 35) and the EPNP-reaction with Asp-32 (refs 29, 36), suggests that in the conditions of the modification reactions, Asp-215 has a higher pK_a (~4.5) than that of Asp-32 (~1.5). The two carboxyl groups as seen in the present crystal structure are not necessarily in the same ionisation state because there are neighbouring side and main chains which interact with the carboxyl groups in a non-symmetric manner. Various hydrogen bonding interactions occur with the Asp-32 carboxyl group which is the first carboxyl group to be deprotonated on raising the pH above 1. The oxygen, of the carboxylate group, which is hydrogen bonded to Asp-215 also interacts with main chain between Thr-216 and Gly-217; the internally buried carboxyl group of Asp-304 interacts with the opposite side of this peptide bond (see Fig. 2). Ser-35 is hydrogen bonded to the other carboxylate oxygen of Asp-32, either directly or through a solvent bridge. There are no equivalent interactions seen for Asp-215 although the side chain of Thr-218 is nearby. In addition, there is an ion-pair (Lys-308 with Asp-11) in the vicinity of Asp-304 and a conformational change brought about by rotation of the carboxyl group of Asp-304 around its C α -C β bond could change the ion-pair to one between Lys-308 and Asp-304. The positive charge of Lys-308 in penicillopepsin is conserved in the mammalian enzymes by having an arginine residue at this position in pepsin and chymosin³⁷. The implications of these interactions which involve conserved residues in all the acid protease sequences thus far determined^{30,37} must await detailed model building of penicillopepsin and a comparison with other high resolution protease structures.

Binding groove

Although we have no direct evidence in the form of difference electron density maps of inhibitor or substrate analog binding, we postulate that the deep groove which separates the two lobes of the molecule is the extended substrate binding site common to acid proteases^{10,26,38}. There are three reasons for this proposal. (1) The two active site aspartic acid residues are located inside the groove and towards one end of it. (2) There is a cluster of three aromatic residues which are very close to the surface of the groove and are located in the larger lobe at distances between

8 and 13 Å from the active site. Trp-39 and Trp-71 have their ring systems stacked and one edge of Trp-39 is in contact with the solvent channel of the groove (see Fig. 6) Tyrosine-75 is also located in this region and is in contact with solvent. Chemical studies with the peptide Leu-Gly-Leu have shown that it binds to penicillopepsin, activates the hydrolysis of Leu-Tyr-NH₂, inhibits the penicillopepsin activation of trypsinogen and produces changes in three of the transition bands (290, 286 and 276 nm) of the CD spectrum of penicillopepsin³⁹. We suggest that the binding of Leu-Gly-Leu and other activator peptides¹⁰ in the groove perturbs Trp-39, Trp-71 (the bands at 290 and 286 nm) and Tyr-75 (276 nm) and that this perturbation on binding causes the circular dichroism spectral changes. There is only one other tryptophan residue, Trp-190, and its circular dichroism band (293 nm) does not change when activator peptides are bound to penicillopepsin. Trp-190 is buried in the hydrophobic core, inaccessible to solvent and removed from the active site region. (3) The deep groove observed in the low-resolution study of the acid protease from *R. chinensis*⁷ is the site of binding of inhibitors to this enzyme. As the other acid proteases have a cleft in the molecule, it is probable that the feature we observe is the extended substrate binding cleft.

Enzymatic studies have led to estimates of the total length of the substrate binding groove of seven⁴⁰ to eight or nine amino acid residues⁴¹. For a fully extended substrate chain this would correspond to a length of 26–32 Å. In penicillopepsin we have estimated the length of the binding site on the N-terminal side of the scissile bond (secondary site A and primary site S; as defined by Takahashi and Hofmann⁴²) and found it to be equivalent in length to a tetra or pentapeptide⁴³ (14–18 Å). From the length of the groove estimated on the model (~30 Å) and the circular dichroism evidence, we conclude that Leu-Gly-Leu binds in the secondary site A (ref. 43) and that this site is located in the deeper and longer part of the groove (to the right of the catalytic site in Fig. 4).

Comparison with other acid proteases

The amino acid sequence comparison in Fig. 3 shows regions of extensive homology between penicillopepsin and porcine pepsin. The overall shape and dimensions of penicillopepsin are very similar to those of the acid proteases of *R. chinensis*⁷ and *E. parasitica*⁸. Insufficient information is available for a detailed comparison with the 2.7-Å structure of pig pepsin⁶, but the reported tracing of the path of the polypeptide chain of pepsin⁶ shows no homology to the structure we report here. Although Fruton¹⁰ has suggested that pepsin and carboxypeptidase A have similar enzymatic mechanisms, the tertiary structure of penicillopepsin is distinctly different to that of carboxypeptidase A, which has an extensive arrangement of 8 strands in mixed parallel and anti-parallel β -sheet conformation⁴⁴. We have compared, visually, the polypeptide chain folding of penicillopepsin

reported here to those of the acid proteases from *R. chinensis* and *E. parasitica*. The three structures are in close agreement, strengthening the argument that the porcine pepsin structure will be found to be similar.

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letters to nature

A conspicuous globule in the Southern Coalsack

THE Southern Coalsack is the finest dark nebula in the Southern Milky Way. At the Royal Observatory, Edinburgh, in October 1976, we studied two photographs taken with the SRC 1.2-m Schmidt telescope (a Unit of the Royal Observatory, Edinburgh) at Siding Spring Observatory, Australia. Centred at α (1975) = 12 h 45 min, δ (1975) = $-63^{\circ}01'$, they show the whole of the Coalsack in all its beauty. The blue plate (Fig. 1) was a 60-min exposure on a hydrogen-sensitized IIIaJ plate, taken through a 2-mm Schott GG395 filter; the near-infrared plate was a 60-min exposure on a water-hypersensitized IN plate, taken through a 4-mm RG715 filter. These plates were taken as part of a more comprehensive study of the Southern Coalsack; this note represents a pilot study of how much can be learned by simple star-counting techniques. Visual inspection of these plates confirms the earlier conclusions^{1–3} that the Southern Coalsack is basically a thin obscuring sheet with an average total visual absorption $A_v = 1.2$ mags. Rodgers¹ derived the distance from the Sun to the Coalsack to be 175 pc.

A globule is a roundish, sharply bounded dark nebula with an angular diameter of a few minutes of arc, seen projected as a 'Loch im Himmel' (William Herschel) against a star-rich background. The present pair of Schmidt photographs show a large number of potential globules. A general catalogue is being compiled of globules identified on SRC Schmidt plates of the Southern Milky Way. (This search is being undertaken at Siding Spring by Drs W. M. Goss, R. Manchester, T. G. Hawarden, A. J. Longmore and I. J. Danziger.)

Westerlund was the first to draw attention to the numerous globules in the Southern Coalsack. His original conclusions have been confirmed and extended by Tapia⁴. The most obscured (star-poor) regions in Tapia's Survey are the regions Tapia I and Tapia III. In our survey we have catalogued 27 globules and patches of dense obscuration associated with the Coalsack, some of which are identified in Fig. 1. The first six objects in our catalogue are the

globules listed in Table III of Tapia's paper. The densest and most nearly perfect of these globules is the second one in Tapia's list, located at α (1975) = 12 h 30.0 min, δ (1975) = $63^{\circ}37'$ in the region Tapia III. It has an angular diameter of about 6 arc min (0.3 pc). We will refer to this as globule 2.

A comprehensive study of these globules awaits *inter alia* the establishment of standard magnitude sequences in B, V, R and I, to $V=22$. Useful information on globule 2 has been obtained, however, from inspection of the two available plates. At the suggestion of Dr R. D. Cannon, and with the assistance of Mr R. D. Eberst (both of the Royal Observatory, Edinburgh) a modified Zeiss Blink Comparator has been used to make a fairly complete study of the optical properties of globule 2 by applying simple star-counting methods. The comparator has been modified by J. M. Allen to incorporate a closed circuit TV system similar to that designed and built by J. G. Bolton at ANRAO, Parkes, N.S.W. In addition to the blink facility, the images on a pair of plates may be viewed on the TV monitor simultaneously or separately, side by side or superimposed, or in split-screen modes, by using the appropriate 'wipe' or 'fader' facilities. In the present case the infrared and the blue plates were inspected in a nearly superimposed mode, with the infrared images appearing as black dots and the blue images as white dots in the TV display (Fig. 2).

Beyond the outer limits of globule 2 each star shows both red and blue images, generally right up to the rather sharp outer edge of the globule at about 3 arc min from its centre, measured on the blue (J) plate. In the annulus lying between 3 and 1.6 arc min from the centre of the globule, 37 stars have red images without blue companions, and only four stars in this annulus have faint blue images. There are no stars visible in either colour within 1.6 arc min of the globule centre. The star counts were done from polaroid photographs of the TV display. These simple observations show that the dust in globule 2 is highly concentrated towards its centre. Similar counts were made in a comparison field in a star-rich region 17 arc min south of globule 2. In this field 186 stars had images on the infrared plate, and of these 170 stars also appeared on the blue plate, in an annulus of radii 1.6 and 3 arc min.

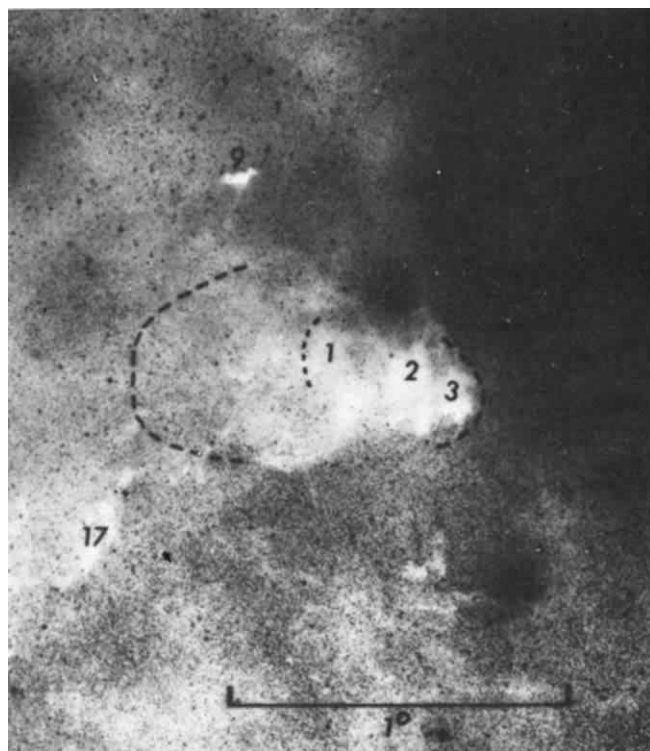


Fig. 1 Part of the Southern Coalsack, reproduced from a deep IIIaJ plate taken with the U.K. 1.2-m Schmidt telescope at Siding Spring. Globules 1, 2 and 3 in Tapia's list are numbered. α Crucis is shown on the right (west) of the print. The long-dashed curve indicates the approximate boundary of the HI absorption observed by Kerr *et al.*⁸, and the short-dashed curve shows the eastern boundary of the formaldehyde absorption observed by Brooks *et al.*⁹.

An approximate minimum dust mass for globule 2 can easily be derived from the star counts using the methods described by Bok, Cordwell and Cromwell⁵ and in the doctoral thesis of Dickman⁶. We base our calculations on the log $A(m)$ against m curves in the blue and near infrared shown by Tapia⁴. These curves were derived from stars between the 12th and 16th magnitude, but the limiting magnitude of the blue Schmidt plate is at least $B \geq 22$. Star-count data to 21.0 mag (ref. 7) indicate that the slopes to be used in our calculations should be approximately two-thirds of those derived by Tapia. Comparisons of the near-infrared star counts in the annulus round the globule 2 and in a comparable area in the comparison field yield a value of the average absorption in the near infrared (about 8,500 Å) $A_I = 2.7$ mag. Corresponding values for A_B and A_V are $A_B = 6.5$ mag, $A_V = 5.0$ mag. The value of A_B is roughly confirmed by the presence of four stars in the annulus which have faint blue images. This is about the number we would expect for $A_B = 6.6$ mag. The values quoted apply strictly only to the annulus between 1.6 and 3 arc min from the centre of the globule.

A minimum dust mass for the entire globule can be obtained by applying the above values to the whole of the globule. The mass thus derived underestimates the total contribution from the core, for which we know only that A_V and A_B must be greater than the values given above for the outer ring. The derived minimum dust mass is $0.05 M_\odot$. Using a factor 200 to proceed from the dust mass to the total mass of dust and gas (presumably mostly molecular hydrogen, H_2), we derive an approximate minimum mass of $10 M_\odot$ for globule 2. This is a minimal value since we have not been able to penetrate the 1.6-arc min diameter core of this globule, which may be quite massive, possibly as high as $25 M_\odot$. The estimate of the minimum total mass should be halved, however, if the dust to gas ratio in this region is assumed to be 1:100. We note that the total absorption A_V enters linearly into the formulae from which the total mass is derived.

Kerr, Hawarden and Bowers⁸ searched for HI self-absorption in 19 regions in the Coalsack, and found it only in the region of

globule 1, 2 and 3. The neutral hydrogen peaks over globules 1 and 2 and tails off for 35 arc min east of globule 1. In the outer edges of the HI distribution the optical absorption is much lower than in other regions where no HI absorption was seen. Brooks, Sinclair and Manfield⁹ have observed the distribution of OH 1,667-MHz emission and formaldehyde 4,830-MHz absorption over the complex. The OH distribution resembles the HI distribution (and hence the optical absorption distribution shown in Fig. 1) while the formaldehyde was detected only in the regions of highest dust density, outside of which it falls off steeply on all sides. Sinclair and Brooks¹⁰ estimated the diameter of the formaldehyde cloud in globule 2 to be 8 arc min, very similar to the optical diameter found from our photographs of globule 2 and its close neighbours.

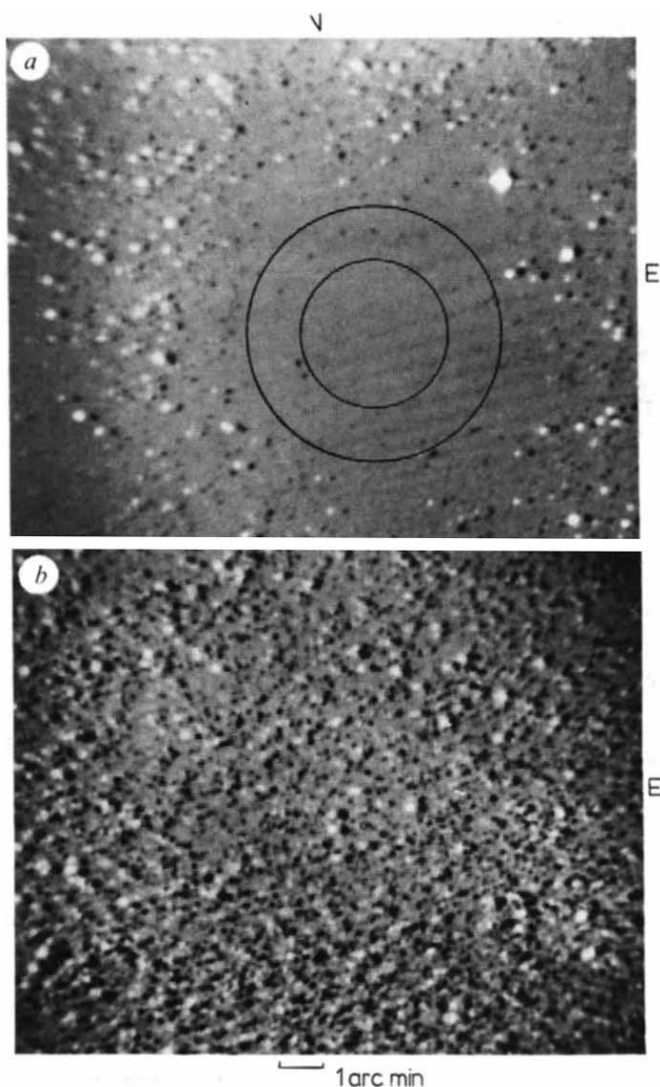


Fig. 2 TV monitor display of *a* globule 2, and *b* the comparison field. Images from the blue plate are shown in white; images from the near infrared plate are shown in black.

Radio observations are beginning to yield much additional information on the physical conditions in the complex around globule 2. A team from Queen Mary College, London¹¹, have used the 3.9-m Anglo-Australian telescope to observe the 2.6-mm line of CO at six points. Their preliminary results show that the CO emission also peaks over globule 2, for which they derive a mass of several solar masses and kinetic temperatures between 6 and 16 K. Dickman's results for globules accessible from northern latitudes would suggest probable numbers of ^{13}CO molecules in globule 2 of between $N_{13} = 10^{16}$ and $N_{13} = 2 \times 10^{16}$. His formula for the mass of the molecular hydrogen in g cm^{-2} is: $N_{\text{mass}} (\text{g cm}^{-2})$

$= 2 \times 10^{-18} \times N_{13}$. From the widths of the lines in the radio spectrum it should also be possible to derive the velocity dispersion of the CO molecules and any rotation of the globule, if present, should be detectable to within $0.1-0.2 \text{ km s}^{-1}$.

The studies of globules by Martin and Barrett¹² and by Tucker *et al.*¹³ show that globules are most likely to be gravitationally bound and in a state of collapse. The smaller ones among the large globules, such as Barnard 335, have radii of the order of 0.3 pc and masses of about $25 M_{\odot}$. Larger objects in this class, such as Barnard 5, have radii of the order of 1.2 pc and masses as high as $800 M_{\odot}$. Globule 2 ranks as being smaller and a little less massive than Barnard 335. The smaller ones will probably collapse into single or more likely multiple stars, whereas those of higher masses may collapse into modest star clusters. Globule 2, as well as most other globules in the Southern Coalsack, is probably on the verge of collapsing into a single or multiple star. The time required for collapse into a protostar (or a modest protocluster) is probably $< 1 \text{ Myr}$.

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Periodic fadings in VHF radio-satellite transmissions during the solar eclipse on 23 October 1976

THE localised time-dependent cooling action of the lunar shadow during an eclipse may generate gravity waves at ionospheric heights^{1,2}. The bow wave thus formed would be detected at great distances from the eclipse path. Experimental data in the northern hemisphere from the eclipse on 7 March 1970, indicated the presence of gravity waves in the F-region, deduced from the incoherent backscatter technique³, vertical incidence soundings⁴, and recordings of the Faraday rotation angle of VHF transmissions from geostationary satellites⁵. The detected travelling ionospheric disturbance (TID) had the horizontal phase velocity of 400 m s^{-1} (ref. 4) and $620 \pm 120 \text{ m s}^{-1}$ (ref. 5). TID was reported to travel north to south¹, or $279 \pm 25^\circ$ east of north³. We report here our observations of large-magnitude periodic fadings during the eclipse of 23 October 1976, which indicate that the eclipse is associated with generation of wave disturbance affecting radio-satellite transmissions in the

upper VHF. The waves had a 40-km wavelength, and propagated from north to south with a velocity of 556 m s^{-1} .

It seems that no experiment has been conducted on the effect of the solar eclipse on the amplitude of radio transmissions from non-synchronous satellites. This was mainly due to the limited number of orbiting satellites used in previous scintillation studies. Thus, the probability of coincidence of an eclipse and a satellite pass was small. Our results are based on amplitude recordings of VHF radio signals from six U.S. Navy Navigational Satellites (NNSS) transmitting at a frequency of 149.988 MHz, and D2-B satellite (catalogue No. 8332) transmitting at a frequency of 136.740 MHz. NNSS and D2-B had orbit inclinations of 90° and 37° , respectively. The recording station was at Brisbane (27.5°S , 152.9°E geog. lat. and long., and 37.2°S invar. lat.).

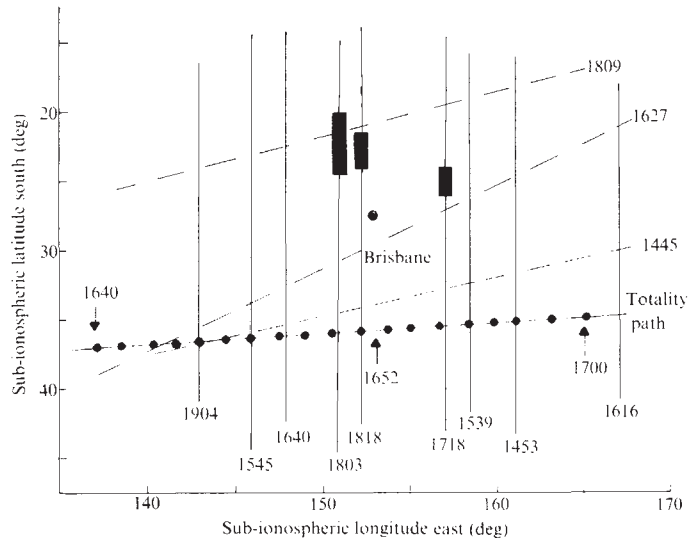


Fig.1 Sub-ionospheric orbits of NNSS (—) and D2-B (---) satellites during the solar eclipse on 23 October 1976. The first periodic fadings (vertical bar) were recorded during a NNSS pass at 1718 LT after the time of the closest approach of totality at 1652 LT. Arrows show the central positions of the eclipse at different local times.

The path of eclipse totality on 23 October 1976, ran south of the Australian continent between about 1600 and 1700 LT (150 EST). It was possible to record a relatively large number of satellite passes during the eclipse period, using radio transmissions from the seven orbiting satellites. Figure 1 shows the sub-ionospheric orbits for NNSS and D2-B at 400-km altitude. The satellite passes at 1445, 1453, 1539, 1545, 1616, 1627 and 1640 LT (times of the predicted nearest approaches) did not show any scintillation activity. A NNSS pass at 1718 LT revealed pronounced periodic fluctuations of a large magnitude (3 dB peak-to-peak) superimposed over normal, slow variations in amplitude (Fig. 2). The disturbed region was north of Brisbane (vertical bar in Fig. 1). Assuming that the disturbance occurred at the point of closest approach of totality at 1652 LT, the velocity of propagation of the waves is 556 m s^{-1} .

The following NNSS passes at 1803 and 1818 LT indicated the presence of periodic fadings of an unusually large magnitude (5 dB peak-to-peak in Fig. 2), confined to the region further north of Brisbane (Fig. 1). The periodic fadings were absent during a pass at 1904 LT. A D2-B pass at 1809 LT from south-west to north-east did not reveal any periodic fluctuations (Fig. 1), indicating that the wave-front was directed more towards the geographic north than east-west, since radio fadings are enhanced in the direction of the steep ionisation gradient generated by the passage of a periodic disturbance. It should be also noted that the largest

periodic fadings were recorded about the time when the eclipse terminated. This may be associated with the enhancement of TIDs at the end of the eclipse due to an interference effect of the waves generated at various positions of the totality. The possibility of such enhancement was suggested in association with the eclipse on 7 March 1970 (ref. 1).

The disturbances shown in Fig. 2 had a typical period of about 10 s, which corresponds to a 40-km wavelength at ionospheric heights. This is considerably smaller than the wavelengths found from the ionospheric experiments conducted during the 1970 eclipse. For example, the total electron measurements³ indicated a 20-min period of the disturbance, which corresponds to a wavelength of about 700 km. This difference may be due to the various spectral characteristics of the techniques used for detection of ionospheric fluctuations; the total electron and satellite scintillation methods tend to indicate long and short scale sizes of the disturbance, respectively. From this, it seems that the eclipse generates TIDs with a number of wavelengths ranging from about 40 to 700 km.

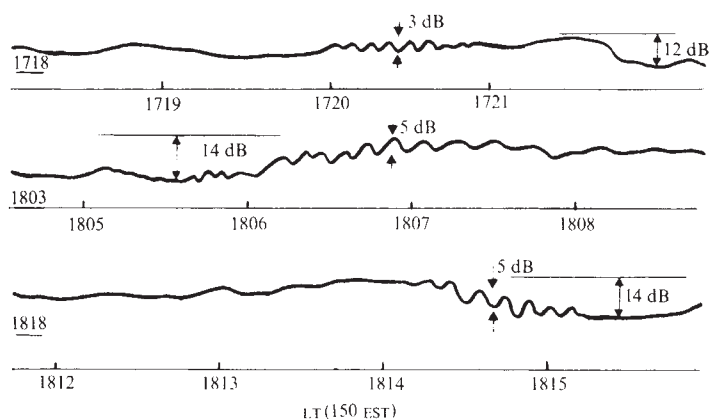


Fig. 2 Amplitude structures of periodic fadings in VHF radio-satellite transmissions during the eclipse period.

The distinct and prolonged periodic fadings (Fig. 2) are an unusual feature of the radio-satellite experiment conducted over several years at Brisbane. Amplitude records obtained from 150 satellite passes in October, 1976, indicated the presence of periodic fadings in 3% of passes. The periodic fadings recorded at other times than the eclipse period had a less well-defined quasi-sinusoidal structure and their magnitude was ≤ 3 dB peak-peak. Also, their duration was limited to one satellite pass only, unlike the events shown in Figs 1 and 2, which were recorded during three consecutive satellite passes.

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Electrons and positive ions in an auroral arc

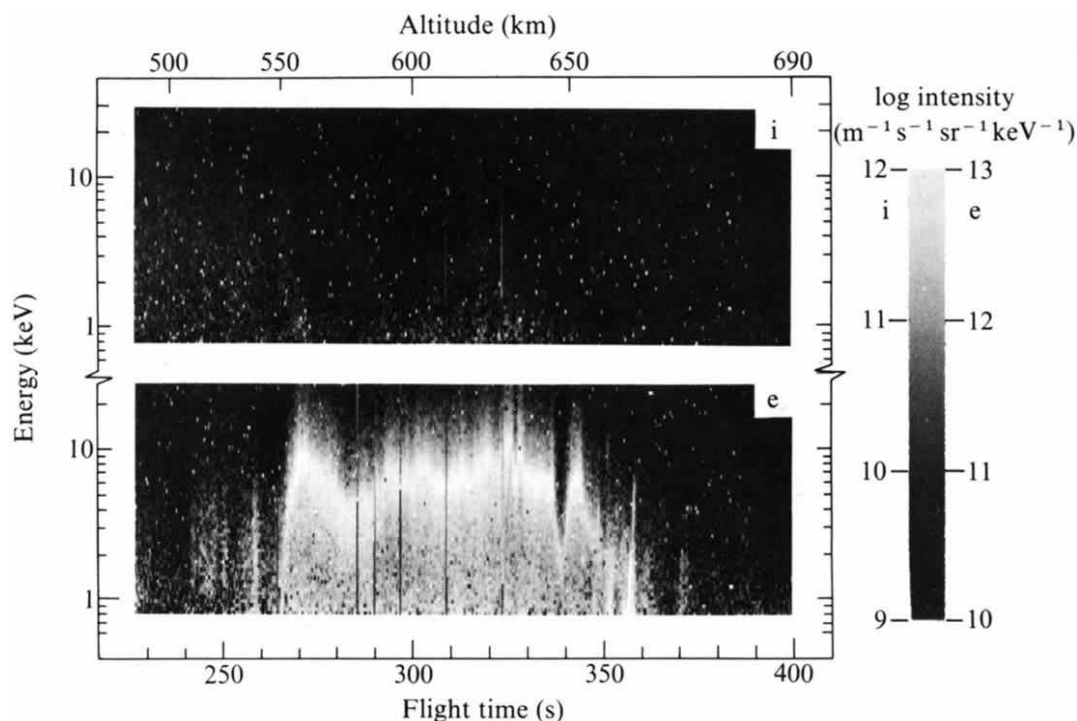
Two new types of British sounding rocket, a Skylark 12 and a Fulmar, were launched from Andøya in northern Norway, on 21 November 1976, in an experiment to study the particle streams and plasma waves associated with an auroral arc. We report here the first results obtained from channel multipliers and electrostatic analysers carried on the Skylark 12 (designation SL 1422) to detect electrons in the range 0.5–25 keV, and positive ions of 0.5–25 keV per unit charge. We believe that the results are unique and constitute the first detailed comparison of positively- and negatively-charged particles in the region of an auroral arc. They have been made possible by the combined advantages of high altitude (715 km), which enables ions to be studied before absorption in the atmosphere, long range (780 km), which allows a complete traversal of an auroral structure, and new analysers, which extend the dynamic range of measurement.

Spectrograms of positive ions and electrons taken from near the middle of the flight are shown in Fig. 1. In this period the rocket, travelling northwards, left the diffuse aurora to the south of the arc to enter at 265 s the intense stream of electrons that produced the luminous east-west curtain of the visible arc. The period shown also encompasses the rocket's emergence at 360 s into a region of sub-visual aurora to the north of the arc.

We note first that the arc is characterised by relatively high electron intensities and energy spectra with a pronounced peak whose position varies within the arc between 2 and 9 keV. We note also the marked anticorrelation between electron and ion intensities during the transition between the diffuse aurora and the arc. The anticorrelation does not persist far into the arc: between 305 and 330 s, although there is little obvious change in the electron intensity, the ion intensity rises to form a spectrum with a broad peak around 6 keV, a minimum near 2 keV, and a tail rising towards low energies. This ion spectrum is unlike those measured between 150 and 250 s which are approximately Maxwellian with a most probable energy of 10 keV. To the north of the arc after 360 s the most probable energy of the ions is 3 keV, and the electron spectrum has a power-law form with intensities decreasing towards higher energies. (Unfortunately, in the presence of unremoved telemetry noise, only some of these features are readily discernible in the black-and-white spectrogram of Fig. 1.)

We offer the following preliminary interpretation of these results based on conclusions drawn from earlier lower-altitude flights about the mechanism for electron acceleration in auroral arcs^{1,2}. To the south of the arc we suppose the electrons and ions to be scattered without significant acceleration from their source plasma, the plasma sheet of the magnetosphere, where electron and ion temperatures correspond to 2 and 10 keV, respectively. The particles forming the arc are from the same source, but in this case the electrons are accelerated during precipitation by the equivalent of a potential difference that fluctuates rapidly about a most probable value indicated by the position of the peak in the spectrum. The fluctuations are invoked^{1,2} to account for the breadth of the peak on the low-energy side; the breadth on the high-energy side is simply a consequence of the finite temperature of the source plasma. Ions, being subject to the same potential differences, are retarded and reduced in intensity when electrons are accelerated. This would account for the anticorrelation near the southern border of the arc. We suggest that the increase in ion intensities near the centre of the arc, where electrons still seem to be accelerated, is an example of an occurrence predicted earlier³, namely, that at times the effective potential difference could fluctuate enough to reverse in sign temporarily and apparently accelerate ions and electrons simultaneously. The peak in the ion spectrum, being sharper than that of the Maxwellian spectra to the south, is consistent with acceleration taking place at this time. The facts that the ion peak is broader than the electron peak and the ion intensity is less enhanced than the

Fig. 1 Positive-ion (i) and electron (e) spectrograms from SL 1422 launched at 2115 UT on 21 November 1976. The intensities were measured at pitch angles in the range 30–40°.



electron intensity indicate a broader distribution of energy gain for the acceleration of positively-charged particles. North of the arc the electrons and ions are of lower energy than those to the south, and the electron spectra are different in form. This supports the suggestion made previously, based on shorter-range flights^{4,5}, that auroral arcs are phenomena occurring at the boundary between the plasma sheet and high-latitude magnetotail regions of the magnetosphere.

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Speckle image reconstruction of solar features

SPECKLE imaging is a process which permits recovery of diffraction limited resolution from atmospherically degraded astronomical images. We describe here the application of this new process to the reconstruction of solar surface features with resolution approaching the telescope limit, despite severe atmospheric blurring in the original data.

Speckle imaging has evolved from speckle interferometry, which has been used to measure stellar diameters¹ and binary stars² to an angular resolution of 0.025 arc s and has yielded evidence of sub-arc s surface detail when applied to solar images^{3,4}. In speckle interferometry only image autocorrelations are obtained, restricting information recovery to centrosymmetric detail. In speckle imaging, true diffraction limited images of extended objects can be reconstructed without requirement of a reference star in the field.

The data-taking requirements are identical for both imaging and interferometry: a series of short exposure, narrow pass-band, highly magnified images are recorded on film. For most low light level astronomical objects, an image intensified camera is used, though for solar work no intensifier is required. The conventional procedure for processing speckle interferometry data is to Fourier transform individual images and sum their power spectra. In the imaging process, the phase in the Fourier transform of each frame is encoded in the form of point-to-point phase differences. This is performed in two dimensions by calculating the shifted complex product of the transform. The results for each frame are summed, yielding averaged quantities $\langle A(u,v) \rangle$, in the form

$$\langle A_u(u,v) \rangle = \langle I(u,v) I^*(u + \Delta u, v) \rangle \quad (1)$$

$$\langle A_v(u,v) \rangle = \langle I(u,v) I^*(u, v + \Delta v) \rangle$$

where $I(u,v)$ is the image Fourier transform and the two averages encode the phase in the u and v directions, respectively. The shifts, Δu and Δv , must be small compared to the resolution element in the object transform.

Each of the components in equation (1) can be expanded in the form

$$\begin{aligned} \langle A(u,v) \rangle \simeq & O(u,v)^2 \exp\{i[\Phi(u,v) - \Phi(u + \Delta u, v)]\} \times \\ & \times \langle T(u,v)^2 \exp\{i[\Psi(u,v) - \Psi(u + \Delta u, v)]\} \rangle \end{aligned} \quad (2)$$

where $O(u,v)$ is the object Fourier transform, $T(u,v)$ is the atmospheric transfer function, and $\Phi(u,v)$ and $\Psi(u,v)$ are the phase in the object transform and the atmospheric transfer function, respectively.

The transfer function term in equation (2) has an expected value whose phase approaches zero (since the atmospheric phase fluctuations are a stochastic, zero mean process) and whose amplitude approaches the diffraction limited transfer function usually obtained in conventional speckle interferometry. For reconstruction, the relative phase at any point in the transform is calculated by summing the phase differences between that point and an arbitrary reference point over a multiplicity of paths, which greatly reduces the effects of noise

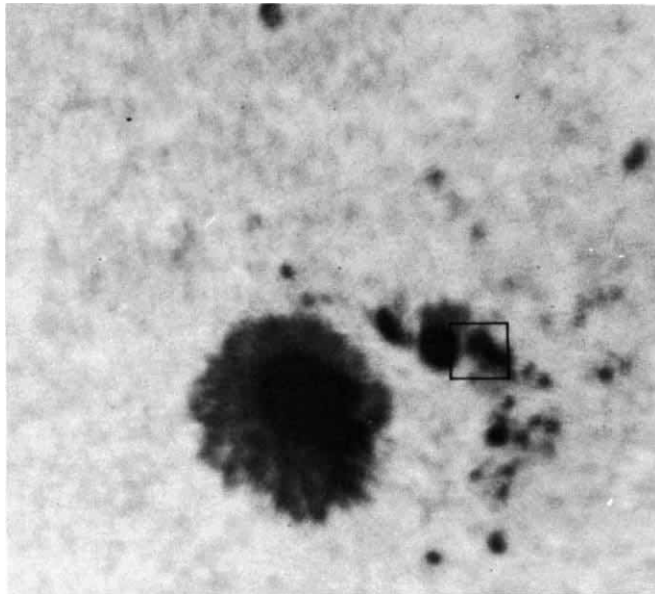


Fig. 1 Photograph of the Sun showing the region processed.

and bad data points. Finally, the output image is obtained by Fourier inversion of the resulting phase and amplitude distribution. This procedure is based on an algorithm suggested by Knox and Thompson⁵ and developed and demonstrated for two-dimensional extended, continuous tone objects with simulated atmospheric degradation by Nisenson *et al.*⁶. This process is unrelated to the technique reported by Lynds *et al.*⁷, which is applicable only to small objects, close to the telescope diffraction limit in extent. It is also unrelated to the large class of image-processing algorithms in which an inverse impulse response is estimated and applied to restoration of the image.

The photographs processed in this experiment were obtained from Dr J. W. Harvey of Kitt Peak National Observatory and were taken at the 24-inch vacuum solar telescope. The passband was 10nm centred on 500 nm and the exposure time was $\sim 1/100$ s. At the magnification used, the telescope diffraction limit occurs at a spatial frequency of about 35 lines per mm, well within the transfer characteristics of the Kodak 5460 film. Thirty-two frames were suitable for processing. Figure 1 is a typical frame covering a 2-min square field. Only the indicated 8×8 -arc-s area, which included a dark feature, was processed. Note the presence of irregular fringe-like interference features in part of the image. These vary randomly from frame to frame, and are coherent phenomena induced by atmospheric turbulence.

On each frame the subarea was scanned and digitised as a 128×128 array centred on a selected feature. A 128×128 array was chosen to allow two samples per resolution element and to restrict the processed region to an area smaller than the expected isoplanatic patch size. The isoplanatic patch is determined by the angle over which the atmospheric point spread function remains highly correlated.

The digitised frames were then processed as we have described. Each frame was digitally Fourier transformed using an FFT algorithm and, after phase coding, the transforms were added together and the phase reconstruction algorithm applied. The phase and amplitude distributions derived from the averaging process are then retransformed to obtain an image.

Figure 2 includes two typical input frames after scanning and redisplay. The seeing limit varies from frame to frame and from region to region in the full field (see Fig. 1); however, the average is about 2 arc s, which is 12 times the telescope diffraction limit.

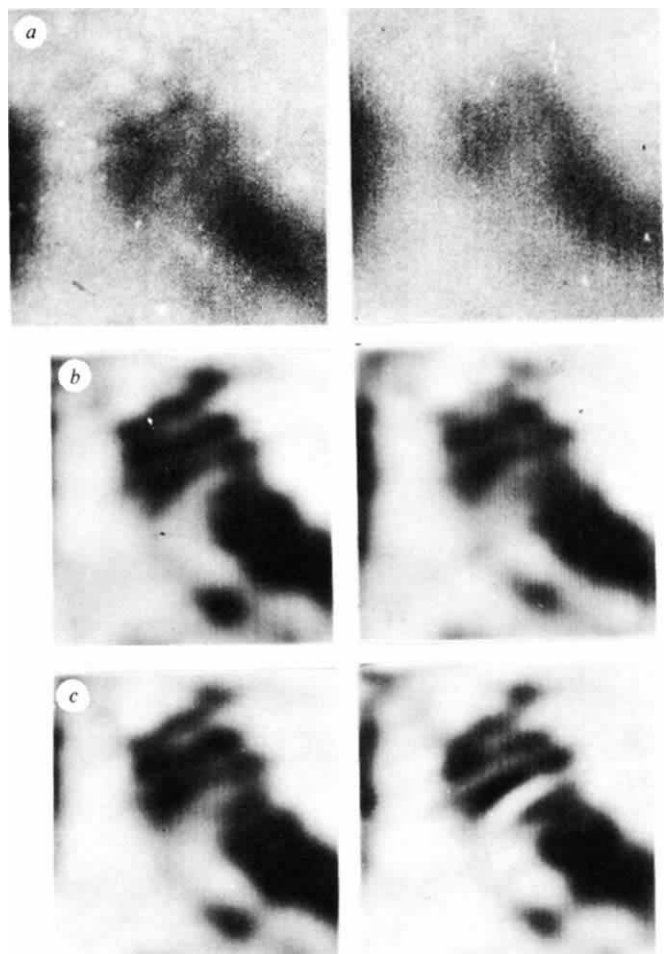
Separate reconstructions produced from 4, 8, 12, 16, 24 and 32 frames show development of small features with progressively increasing sharpness. Reconstructions produced from sequences of frames selected to be free of defects were also nearly identical to those obtained without selection. Figure 2b

shows two independent 12-frame reconstructions, demonstrating that the essential features are real. Figure 2c shows 24-frame and 32-frame reconstructions. The resolution in the final processed image is better than 0.4 arc s and some features such as the bright lanes in the filamentary structure near the centre of the image may be closer to 0.2 arc s, the telescope limit. The resolution (0.4 arc s) is comparable with that obtained in the Stratoscope balloon experiments.

Processing a smaller subarea may have the effect of increasing the rate of convergence to a diffraction limited image if isoplanatism is a problem. The size of the isoplanatic region for solar observations can only be estimated, and a processed area as large as 8×8 arc s may not quite converge to the diffraction limit with only 32 frames.

The processed subarea contains a feature which, from the large scale photograph, would be described as a medium-sized pore. In the reconstruction, the region near the centre of the frame breaks up into a number of dark, parallel, elongated features reminiscent of penumbral structure, while the region below it retains the appearance of a typical pore. The penumbra-like structure is approximately radial to a large nearby spot although it is not connected to it. At least two features looking like very small pores approximately 0.7 and 1.0 arc s in size are detached from the primary structure except for a dark lane. Other dark lanes are evident in the picture, the longest extending about 3 arc s.

Fig. 2 a, Two typical unprocessed frames (8×8 arc s) used in producing the reconstructions after scanning and redisplay; b, two reconstructions produced from independent 12-frame subsets of the data; c, 24-frame (left) and 32-frame (right) reconstructions. Resolution is approximately 2.0 arc s in the unprocessed frames and approximately 0.4 arc s in the processed frames.



The lighter regions of the reconstruction show evidence of photospheric granulation. This is particularly interesting because there is virtually no evidence of this structure in the original digitised frames, partly because of the limited dynamic range of the scanning instrument, which was set to scan optimally the spot region. The original negatives of the reconstructions show that the entire photospheric region is covered by granules, the smallest of which are less than 0.5 arc s in diameter.

To obtain wide field reconstructions, individual isoplanatic regions must be processed independently and combined in a mosaic. This is a lengthy process that requires large amounts of computer and scanner time. To solve this problem, an optical processor that performs an analog of the digital process is now being developed³. Optical processing allows encoding of the phase and direct summation of the individual transforms. The output of the processor is a single frame that is scanned and digitised for computer reconstruction of the final image. With this system, a number of contiguous isoplanatic regions can be processed in parallel, yielding images of relatively large areas at once.

In conclusion, we would like to point out that the ultimate resolution of the process is the diffraction limit of the telescope, independent of the actual seeing conditions. Data taken on the 60-inch solar telescope at Kitt Peak, for example, could display features down to 1/15 arc s, and this can be extrapolated to even larger apertures.

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Long term solar activity forecasting using high-resolution time spectral analysis

SOLAR activity as measured by sunspot number has been carefully observed since 1749. It seems to have an 11-yr cycle which has been identified as a 22-yr magnetic period with every other 11-yr cycle having a reversed average solar magnetic field. As the amplitude and frequency of the cycle varies slowly with time it has become a point of interest to determine if sunspot activity variation is regular and constant over long periods of time. If it is constant, then it should be possible to make predictions based on historical observations. I show here that the monthly sunspot record (1749-1975) is represented by a Fourier Series, using the spectral analysis method of A. Paul. By

evaluating the series, a forecast indicates that the next solar cycle began in December 1976, and will peak above 100 near 1982.

Eddy¹ has pointed out that solar activity may have been greatly different during the Maunder Minimum (a period of very low sunspot activity between 1645 and 1695) than has been generally assumed. For example, Schöve² relied upon the assumption of nine cycles per century to identify solar maxima back to the fifth century BC. Eddy discounted Schöve's estimates of the solar maxima magnitudes during the 1600s as systematically high, noting that the magnitudes were sometimes based on "auroral counts and other unspecified data". Eddy stated "the present cycle of activity may be unusual if not transitory".

On the other hand, Wolff³ discussed physical processes within the Sun from which it is reasonable to expect long-term persistence of discrete line spectra in the sunspot activity record. He has developed a rigid and differential rotation model whose modes have beat frequencies which are mathematically related to each other. Using only two adjustable frequencies in the model, he predicted 20 of the 25 periodicities observed in the sunspot data by Cole⁴ and Currie⁵. The longest period found by Wolff is 178 yr. He considered the difference between the value of 178.0 yr and the 190-yr period found by Cole⁴ to be insignificant since the uncertainty³ in an estimate of a frequency present in a record of length T is commonly given as $\pm 1/(4T)$. Cohen and Lintz⁶ have used a 179-yr periodicity, with several other periodicities, to produce a prediction of solar activity to the year 2050. (Of course, an extrapolation of their model backwards in time would not produce the Maunder Minimum, particularly since they use a d.c. component of 47.4 smoothed sunspots in their model.)

Several methods for analysing spectra have been applied to the sunspot record. Cole used the fast Fourier transform (FFT) to analyse the autocorrelation function and the solar cycle phase data of Schöve and obtained the power spectral density as a smooth continuous function of frequency. Both Cohen and Lintz, as well as Currie, used the maximum entropy method (MEM) to find the smooth continuous power density function. The FFT and MEM methods estimate the frequency of a suspected spectral line at the point of peak power.

I re-examine here the sunspot record from 1749 to 1975 using a high-resolution spectral analysis to determine the frequencies of the periodicities, and the amplitude and phase of each line. The analysis is performed with 2724 monthly sunspot values given by Vitinskii⁷ and by *Solar Geophysical Data*. The algebraic sign of alternate cycles' sunspot numbers is negative to conform to an assumed 22-yr magnetic reversal cycle.

My analysis is an extension of a method developed by A. Paul⁸, which is a very accurate and sensitive method for determining frequencies, amplitudes and phases of unknown lines in a spectrum consisting of both broadband noise and discrete lines. Paul's method differs from conventional FFT methods in that it does not assume that frequencies will be uniformly spaced. The time shift theorem ($\Delta\phi = 2\pi f\Delta t$) is used to determine the unknown frequencies from the change of phase between the periodogram analysis of segments of the data separated in time.

My analysis uses the additional step of subtracting the waveform corresponding to the approximately known lines in the data, following by iteratively solving for closely spaced lines. The analysis applied to the sunspot record obtains resolution of closely spaced lines having a beat period $(1/\Delta f)$ of $1,045 \pm 200$ yr. The accuracy of each frequency is more a function of the white noise within the filter bandwidth used in the analysis than of the record length. In this case, the frequency uncertainty is estimated to be $\pm 1/(207)$ rather than the commonly quoted $\pm 1/(4T)$. The success of the line resolution just mentioned results

from the signal-to-noise ratio (line amplitude to Gaussian noise within the bandwidth) of about 25:1.

The model is composed of more than 40 lines (Fig. 1) which were determined using more than 100 iterative solutions for one line at a time with all the other lines subtracted from the data. The s.d. of the residuals is 17.0 (unsmoothed monthly sunspots), which is an indication of white noise. Assuming it is uniformly distributed in frequency, the noise within the bandwidth near each line is about 0.6. This has been confirmed by spectral analysis of

The fact that the summed series fails to fit the data as accurately in the first and last cycles as in the central cycles may be an artefact of the data window or it may indicate changes in the physical processes of the Sun, for example, changes in the oscillation mode excitations. Figure 3 extends the model sunspot numbers back to 1000 AD and compares with the ^{14}C level given by Suess⁹. The model fails to reproduce the Maunder Minimum, but does show a 1000-yr period envelope, as discussed by Eddy. The closely spaced lines with periods of 21.62, 22.12 and

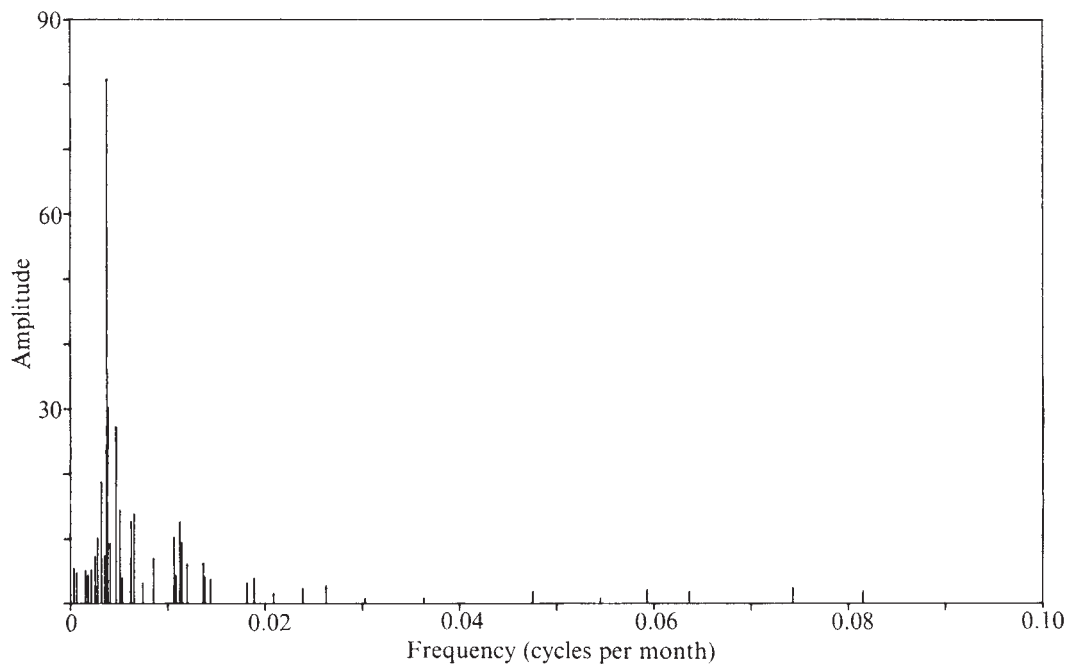


Fig. 1 Spectral lines determined from 227 yr of sunspot data.

the residuals. The effect of the noise is difficult to determine; however, the noise certainly degrades the accuracy of the line parameters.

The summed Fourier series corresponding to the lines in the model is plotted with the monthly data record in Fig. 2. The series is evaluated beyond the data record at both ends to provide a forecast of future and an aftcast of past solar cycles. Note the striking agreement between the model and observations made between 1740 and 1975.

22.55 yr form a triplet with a beat period of about 1045 yr, having maxima (1112 and 2155 AD) during the same centuries as mentioned by Eddy (about 1175) but a minimum (1724 AD) later than Eddy's (1500–1714 AD). The triplet's minimum is not midway between the adjacent maxima due to the particular phase relationships between the three lines.

Although these results are purely empirical, amounting to data fitting, the stability of the solution and the

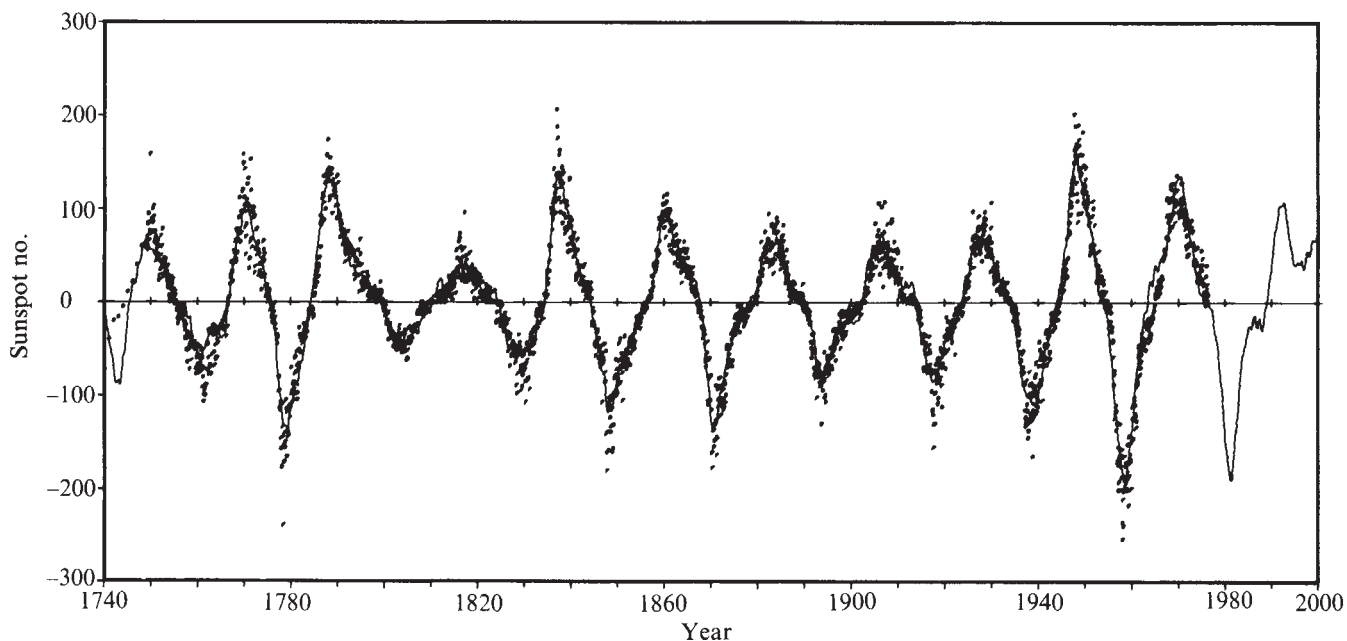


Fig. 2 Observed monthly sunspot numbers (●) and Fourier series model (smooth curve).

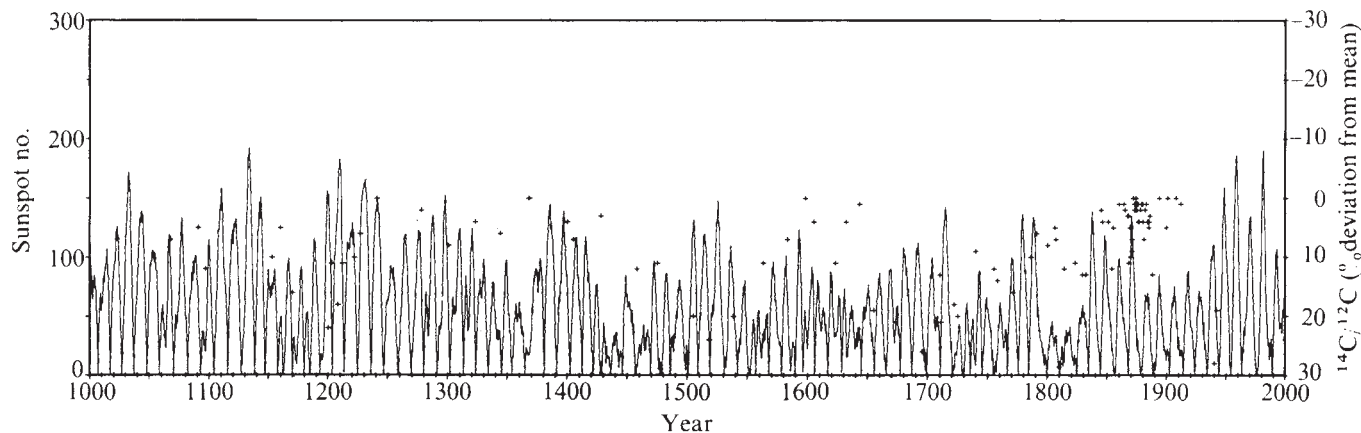


Fig. 3 Fourier Series sunspot model (smooth curve) and $^{14}\text{C} : ^{12}\text{C}$ ratio (+, using an inverted scale).

accuracy of the predictions may be an indication of whether Wolff's mode model is correct. My results show that there are lines in the sunspot record as indicated in Wolff's theory. The modes are long-lived, but may persist for centuries instead of millennia. This latter conclusion can be drawn if we attribute the spectral line model's failure to display correctly the Maunder Minimum to changes in physical processes in the Sun.

One area of possible improvement in the analysis is in the choice of data to represent solar activity. Eddy, Wolff and others have used sunspots, which have been counted for a long time. The noise level in the sunspot data (s.d. of 17) is about as expected, however, considering that sunspot groups are arbitrarily assigned a value of ten spots. Another index of solar activity, such as sunspot area or magnetic field intensity, may be a better quantitative measure. Seventy-five yr of high-quality data might yield better results than spectral analysis of 200 yr of lesser quality data.

The fit (Fig. 2) to the sunspot record between 1750 and 1975 is good enough to merit a forecast a few years into the future. Using the zero crossing as an indicator, the model predicts that the next solar cycle began in December 1976. Several authors^{4,6,10} have predicted a low sunspot activity level during the next cycle (number 21) with a peak near 50–60. My analysis predicts a larger peak between 130 and 200 during 1981 or 1982. It will be interesting to watch the Sun during the next few years to find out for how long the spectral lines of the model are valid.

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The advancing front of a spreading liquid

A WETTING liquid makes a zero contact angle to a solid, and a drop will spread completely over the surface. There are two stages in the spreading process^{1,2}. Initially, the advancing front is pushed from behind by the hydrostatic head of the liquid. As the drop thins out this becomes unimportant and the front is pulled along by the unbalanced surface forces near the perimeter. The shape of the advancing front is not easy to observe without special lighting. In addition, for most liquids it cannot be seen unless precautions are taken to minimise evaporation. I report here microscopic observations which show that the advancing front has a remarkable structure. The moving front does not retain the shape of the original drop. Instead, depending on the liquid, it either assumes a scalloped periodic structure or else moves by a series of random advances, resembling the locomotion of an amoeba, but still with some approximate periodicity.

Figure 1 shows the advancing front for absolute ethyl alcohol spreading on the surface of aluminium that had been evaporated on a glass substrate. (Lighting and precautions for minimising evaporation are described in the legend.) A thin layer of liquid, 1,000–2,000 Å thick, moved ahead of the drop by as much as 1 mm. The drop followed, making a contact angle of about 0.1° to the precursor layer, as determined from the spacing of interference fringes. The front of the thin layer maintained a periodic, almost sinusoidal form as it advanced. This mechanism of spreading seems to be quite general. Similar results were found for a variety of liquids spreading on aluminium—*isopropyl alcohol*, *n*-heptane, toluene, *n*-octane (all reagent grade) and dimethyl silicone oil (molecular weight 340).

These liquids are all volatile and vapour transport may be an important part of the mechanism by which they spread. Observations were also made of the spreading of a non-volatile liquid, a high molecular weight hydrocarbon-substituted silicone oil, General Electric SF-1147 (mean molecular weight 2,000). It is so non-volatile at room temperature that effects of vapour transport are almost certainly absent. Measurement of the rate of evaporation in vacuum showed that less than one monolayer of molecules evaporates per day at room temperature. In air the evaporation would be even slower. The oil spreads on the surface of evaporated gold. The shape of the advancing front is shown in Fig. 2. Here the spreading was considerably slower than for alcohol due to the difference in viscosities (0.5 P for the oil and 0.014 P for ethyl alcohol). As the liquid spread, convex bulges develop at seemingly random points along the perimeter of the original round drop. These then move ahead and smaller protuberances develop. Though there was considerable randomness, it seems that there is an approximate periodicity here, as before with the alcohol. Again the front advanced far ahead of the bulk of the drop and, after several hours, covered all, or nearly all, of the solid surface.

These observations show that the spreading of a liquid on a solid is a complicated process. Typically, a thin layer moves out away from the rest of the drop, pulled by the unbalanced surface forces near the perimeter. This feature has been noted before³ from observations of the drop profiles of spreading polymers. The periodic structure indicates an instability. This may be related to the Rayleigh-Taylor instability^{4,5} that arises when two immiscible fluids are stratified with the denser fluid above. The Marangoni effect⁶ also gives rise to instabilities but it depends on substantial variations of temperature or composition within the fluid, neither of which seems to be present here.

The following simplified picture may contain some of the correct physics. Consider the unbalanced surface force $\Delta\gamma$ at the liquid front

$$\Delta\gamma = \gamma_{SV} - \gamma_{SL} - \gamma_{LV} \quad (1)$$

The components are the familiar interfacial free energies for the solid-vapour, solid-liquid, and liquid-vapour interfaces. The force acts on the liquid near the interface and pulls it out over the unwetted surface of the solid. Its magnitude is proportional to the length of the liquid-solid-vapour line. In a part of the film of thickness, z , assume that, due to some local inhomogeneity of the solid surface, the front develops a semicircular convex bulge of radius R . The total force pulling outward on the semicircle is $\pi R \Delta\gamma$, whereas before the bulge developed, the force on the same liquid was only $2R \Delta\gamma$. The accelerating force is growing more rapidly than the resisting viscous force and there is a tendency for the front to become as convoluted as possible to take advantage of this. The counter-force is the excess pressure, $\Delta p = \gamma_{LV}/R$, exerted by a convex curved surface. The pressure, Δp , is exerted over an area $\pi R z$ and acts to oppose the tendency toward greater convolution. The two opposing tendencies balance when

$$\pi R \Delta\gamma = \pi R z \gamma_{LV}/R \quad (2)$$

This then determines the stable radius of curvature for the undulations in the advancing front

$$R = z \gamma_{LV}/\Delta\gamma \quad (3)$$

If this is correct then it may be possible to make a direct

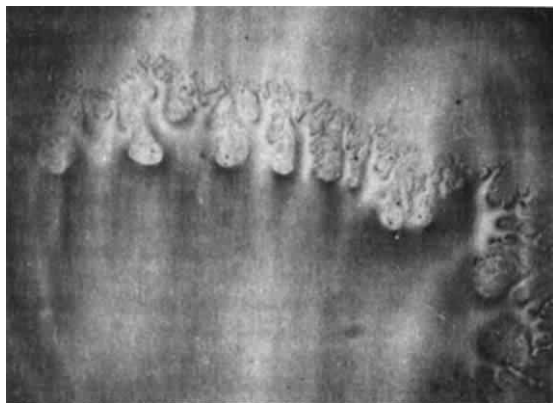


Fig. 1 Advancing front for a drop of ethyl alcohol spreading on the surface of evaporated aluminium. To minimise evaporation the experiment was performed in a cylindrical glass container covered by a glass plate. The bottom of the container was filled with alcohol and the substrate mounted about 1 cm above the surface of the liquid. To apply the spreading drop, the cover was moved aside just far enough to insert a long dropper that reaches nearly to the substrate surface. A drop of about 10^{-3} cm^3 of liquid was dispensed on to the surface and the cover replaced. The drop then spread and the thin advancing front remained stable for many minutes. Vertical illumination with nearly monochromatic light at $5.830 \text{ \AA}$ was reflected from the metal of the substrate to give interference fringes indicating the thickness of the oil. Successive fringes correspond to a thickness difference of $2,100 \text{ \AA}$. The three pictures are for different times after the application of the drop. *a*, 30 s; *b*, 60 s; *c*, 200 s. At *c* the thickness of the advancing front is about $1,000 \text{ \AA}$.

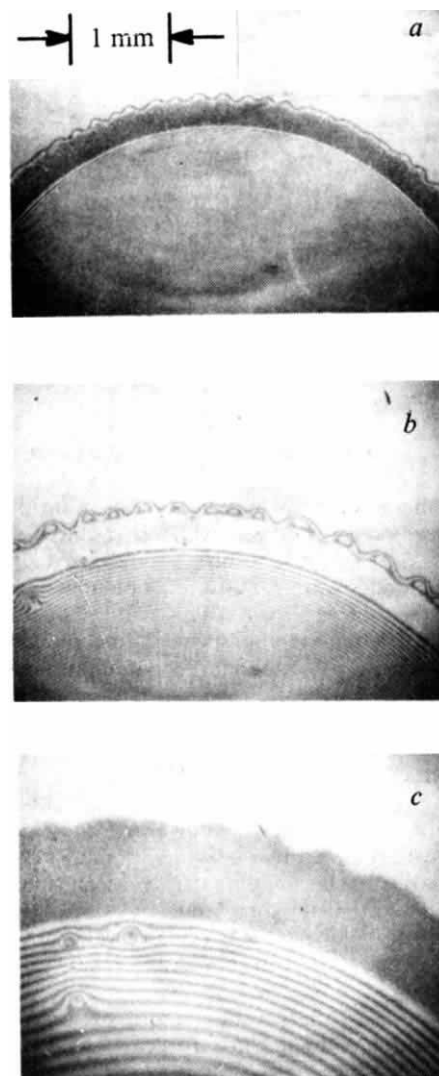


Fig. 2 Advancing front of silicone oil 5 min after application.

measurement of $\Delta\gamma$ since all the other quantities in the equation can be measured.

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Silane triols of high stability in aqueous solution

ORGANO-SILICON compounds containing silanol groups are highly reactive, undergoing acid- or base-catalysed condensation to form siloxanes even in the presence of surface acid or base, the condensation being more facile in the order

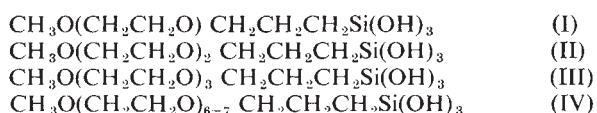


By the use of plastic, quartz or washed borosilicate glass

apparatus, several alkyl and aryl silanols and silane diols have been isolated¹⁻³. But only aromatic silane triols ($R_3Si(OH)_3$) have been isolated³, the corresponding alkyl derivatives being known only as transient species in solution. We report here that certain organo-silane triols display outstanding stability in aqueous solution.

The rate of condensation and polymerisation of silicic acid and organo-silanol derivatives in aqueous solution can be reduced by the addition of polar molecules such as ethers, alcohols and amines and of polymers containing similar groups⁴. The retardation is believed to be due to association through hydrogen bonding between silanol groups and the polar additive. Thus, polyethylene glycols retard the condensation of silicic acid, although inevitably, polymerisation proceeds to the point of gelation or the separation of colloidal silica.

We have prepared silane triols (I-IV), $R_3Si(OH)_3$, in which R is an ether-containing moiety, by hydride insertion of the appropriate halo or alkoxy-silane into the allyl ethers of methoxy ethanol, methyl ethylene glycol, methyl trigol and so on, followed by hydrolysis.



The behaviour of the parent trichloro silyl derivatives of (I-IV) on contact with water revealed notable differences from simple alkyl halosilanes such as CH_3SiCl_3 . The latter in water undergoes rapid hydrolysis with evolution of HCl, generation of heat and the rapid precipitation of a gel—essentially methylated silica gel. This process is little affected by the presence in the water of, for example, $CH_3O(CH_2CH_2O)_2CH_2CH_2OH$. By contrast, the ether-containing compounds dissolve to give clear solutions for which, in spite of the low pH and raised temperature, low levels of polymerisation are indicated by

Fig. 1 Number average molecular weights (cryoscopy) of compound (III).

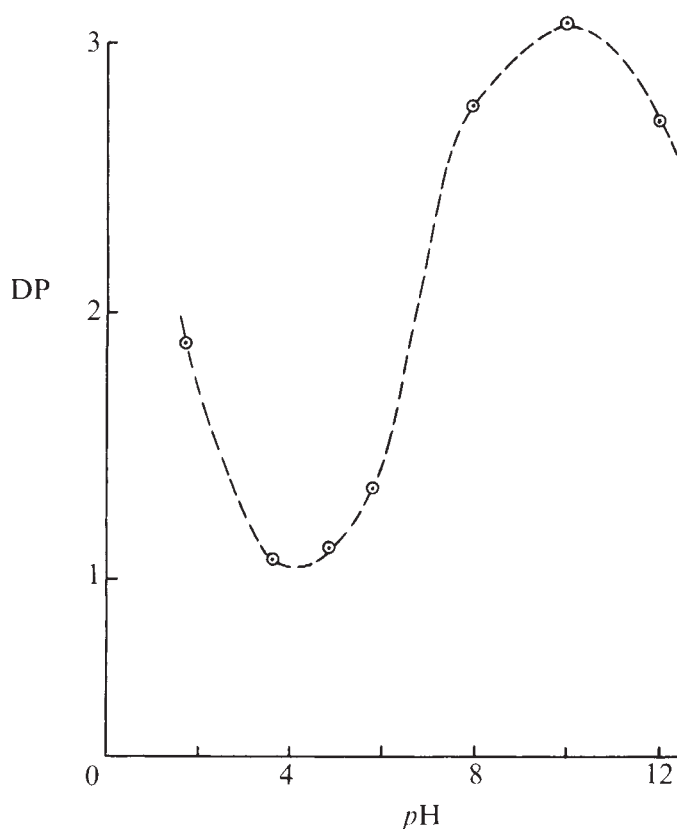


Table 1 Influence of number of ethylene oxide units on stability

Silane triol		Equilibrium DP in 5% (w/w) solution		
		pH 1	pH 5	pH 8
$CH_3O(CH_2CH_2O)CH_2CH_2CH_2Si(OH)_3$	(I)	7.2	2.4	7.1
$CH_3O(CH_2CH_2O)_2CH_2CH_2CH_2Si(OH)_3$	(II)	—	1.9	—
$CH_3O(CH_2CH_2O)_3CH_2CH_2CH_2Si(OH)_3$	(III)	1.9	1.1	3.4
$CH_3O(CH_2CH_2O)_{6-7}CH_2CH_2CH_2Si(OH)_3$	(IV)	2.9	1.1	1.6

DP, degree of polymerisation.

cryoscopy, cloud point and ^{29}Si nuclear magnetic resonance (NMR).

Figure 1 shows the number average molecular weights (cryoscopy) of (III) in 5% (w/w) aqueous solutions as a function of pH within the range 2–12 (after several days equilibration) when the highest degree of polymerisation found was only about 3.

The equilibrium molecular weights for (I-IV) (5% in water) at various pH values (Table 1) clearly illustrate that (I) and (II) are more susceptible to polymerisation than (III) and (IV), which contain the more ether groups.

The cloud point of a 5% solution of (III) did not change from the original value following 4.5 h of heating at 92 °C, whereas that of a 5% solution of (I) or (II) showed a reduction, indicating an increase of molecular weight.

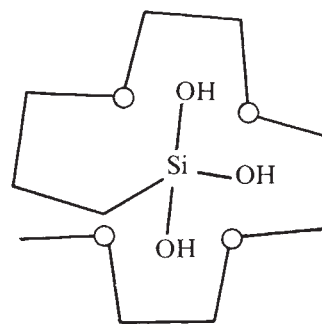


Fig. 2 Possible configuration to give stability.

The essentially monomeric nature of (III) in water was confirmed by ^{29}Si NMR. At pH 5–6 a 5% (w/w) solution contained only a trace of dimer while a 50% (w/w) solution still contained 75% monomer and only 25% dimer with no oligomers. An aqueous solution of $CH_3O(CH_2CH_2O)_3CH_2CH_2CH_2Si(OH)_3$ (III) evaporated to dryness gave a clear, viscous, water-soluble oil with a degree of polymerisation (cryoscopy) of about 7. ^{29}Si NMR showed this oil to contain no monomer or dimer and to undergo slow depolymerisation in water over several weeks.

The results suggest that by incorporating an ethylene oxide function by means of an Si-C bond into a silane triol, high solution stability can be attained. At pH 5, for example, at which the polymerisation of a silane triol is minimal and hydrogen bond formation near-optimal, the equilibrium degree of polymerisation reaches unity when four ether-oxygen atoms are involved. This stability could arise from the fact that the oligomers are soluble and hence, equilibrium would be in favour of depolymerisation. Compounds (I-IV), however, are all adequately soluble for this to apply and yet degree of polymerisation becomes unity only with (III).

This suggests that stability arises from full shielding of the silanol groups by intramolecular hydrogen bonding with the ether-oxygen atoms. A possible configuration (ignoring interposed water molecules) is shown schematically in Fig. 2. A stable, associated pair can also be envisaged in which the silane

triol head group of each molecule is embedded in the ethylene oxide chain of the other.

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Palaeomagnetic field intensity variation recorded in a Brunhes epoch deep-sea sediment core

DEEP-SEA sediments have been shown to possess a natural remanent magnetisation (NRM) that often can be attributed to the statistical alignment of detrital magnetic grains in the Earth's magnetic field at or shortly after the time of their deposition^{1,2}. In favourable circumstances this remanence can be interpreted as a record of palaeomagnetic field behaviour^{3,4}. In the study reported here we have attempted to describe relative variations in palaeomagnetic field intensity on a time scale of 10^4 – 10^5 yr, during the past 700,000 yr, using the palaeomagnetic record of deep-sea sediment piston core RC10-167 (33°2'N, 150°23'E), which has an exceptionally thick section of sediment deposited during the Brunhes normal polarity epoch⁵ (Fig. 1). After subtracting the stratigraphic contribution of several distinct volcanic ash layers interspersed with the otherwise uniform pelagic sediment⁶, we calculate an average deposition rate of 2.1 cm kyr⁻¹ between the adjusted level (1,470 cm) of the Brunhes–Matuyama boundary ($t=700,000$ yr) and the top of the core, assumed $t=0$ yr. A 2-cm thick sample, representing about 1,000 yr of deposition, was taken at an average interval of 3.3 cm (representing about 1,600 yr). This sampling placed a theoretical limit of 3,000–4,000 yr on the period of a resolvable sinusoidal variation.

Progressive alternating field (AF) demagnetisation of the NRM in 12 pilot samples showed consistent directions of magnetisation, to within 10° of the initial value, with demagnetisation up to 400 Oe. The mean remanent inclination after 150-Oe AF is 51.2°, close to the expected dipole inclination (52.4°) for the core site latitude. The median demagnetising field (MDF; peak AF required to remove half the initial remanence) is high and relatively uniform, averaging 270 ± 40 Oe (s.d.). The total magnetic coercivity distribution was investigated by progressive AF demagnetisation of an anhysteretic remanent magnetisation (ARM; imparted in a 1.0-Oe direct field coaxial with a 1,000-Oe AF) and an isothermal remanent magnetisation (IRM; imparted in a 1,000-Oe direct field). The average MDF values for the 12 specimens are 230 ± 15 Oe for ARM and 170 ± 25 Oe for IRM.

On the basis of these experiments we conclude that: (1) the NRM has a high magnetic stability with little contribution from unstable magnetic components; (2) the small scatter in MDF values for NRM, ARM and IRM shows that the magnetic coercivity spectrum is quite uniform over the length of the core; (3) the high stabilities of remanence and the relative stabilities of ARM and IRM⁷ are indicative of fine-grained magnetic carriers; (4) the

coercivity spectrum of NRM is more closely approximated by ARM than by IRM in this sediment.

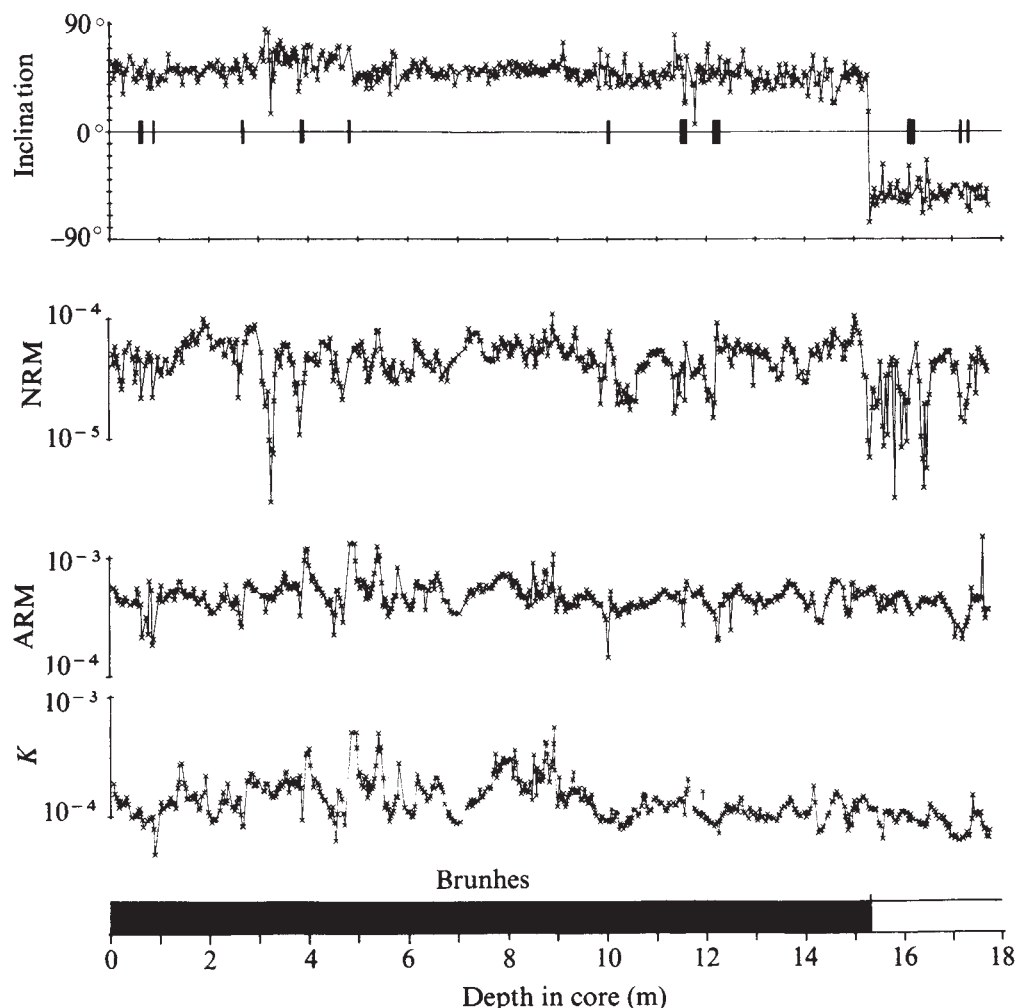
We assume that the magnetisation acquisition process was the same at each stratigraphic level in the sediment and that the intensity of NRM is only a function of palaeofield strength and the magnetic mineral concentration. The variation with stratigraphic level in the sediment (hence age) of NRM intensity, normalised to take into account fluctuations in magnetic mineral concentration, then might provide a record of relative variations in palaeomagnetic field intensity. We do not deal explicitly with the still poorly understood details of the magnetisation acquisition process in the sediment, and so absolute values of palaeofield strength are not obtained directly by this method. A similar approach has been used to describe palaeofield intensity variations across magnetic polarity transitions in deep-sea sediment cores^{8,9}.

Because of our conclusion (4) above, we have chosen to use the intensity of ARM as a relative measure of the quantity of magnetic mineral carrying the NRM. The variation with depth in this core of ARM, however, is similar to that of initial susceptibility (Fig. 1). Together they provide an internally consistent record of fluctuating magnetic mineral concentration. This correspondence supports our inference of a coercivity spectrum that is uniform over the core length since the magnitude of ARM and initial susceptibility each are affected preferentially by different magnetic grain size ranges. By assuming that over this sediment core the intensity of NRM is uniformly and directly proportional to the strength of the magnetising field^{1,10} and to magnetic mineral content as measured by ARM¹¹, the ratio of NRM/ARM should be directly proportional to palaeofield strength alone. In practice, we have used the ratio after partial demagnetisation of each remanence in 150-Oe AF, to remove any possible unstable magnetic components.

A plot of the ratio of NRM/ARM (partially demagnetised) in the Brunhes section of core RC10-167 is shown in Fig. 2. The data have been interpolated linearly to 512 equally-spaced values (from the 448 samples measured), giving an interpolated interval of 2.9 cm (or about 1,400 yr). This was done to facilitate further analysis of the data. The ratio of NRM/ARM has a variation of as much as 100%, but more typically about 50%, about a mean value of 0.10 in the core. The record is nearly stationary as the linear trend is only -0.0036 for 1,470 cm (or 700,000 yr). Visually, three wavelength components seem to dominate the record: a broad baseline fluctuation with an apparent wavelength of about half the record length, a variation with an apparent wavelength of about 1 m which gives the record its dominant character, and last, a short wavelength component that seems to ride on the longer wavelength variations.

A power spectrum analysis was performed on this record to identify better any periodic components. Power spectra were estimated using a direct (fast Fourier transform) method with a cosine-rectangular window, and finally smoothed with a three-point Hanning filter¹² (Fig. 2). We observe an initial falloff in power to a wavelength of approximately 25 cm beyond which the spectrum is flatter and approximates the spectrum of white noise. This change in overall slope in the power spectrum may be a function of the remanence acquisition mechanism in these sediments, the periodic content of geomagnetic intensity variations or a combination of these. For example, deep-sea sediments may not acquire a stable remanence at the instant of deposition but rather at, or more importantly, over some depth below the sediment–water interface^{1,2}. The record of palaeomagnetic field variations with periodicity of the order of the time in which stable remanent magnetisation is acquired will tend to be obscured. On the other hand,

Fig. 1 Inclination direction and intensity of natural remanent magnetism (NRM) against depth in deep-sea sediment core RC10-167. Change in sign of inclination at 15.3-m depth is interpreted as the Brunhes-Matuyama magnetic reversal boundary⁵. Solid bars on axis of inclination plot are the positions of volcanic ash layers⁶. Also shown is the intensity of ARM and the bulk susceptibility (k). The direction and intensity of NRM and the intensity of ARM are after AF demagnetisation in 150 Oe. Magnetisation units are in c.g.s. g^{-1} .



sediment cores with higher deposition rates will need to be studied to determine the contribution of geomagnetic field intensity variations to the shorter wavelength (less than about 12,000 yr) portion of the spectrum.

The power spectrum is dominated by periodic components with wavelengths of several metres and greater, and a component that forms a more coherent peak centred at a wavelength of about 90 cm. We hesitate to ascribe the longer wavelength variation in NRM/ARM to that of palaeomagnetic field intensity. Our record is too short to evaluate such an apparent long period variation and it may reflect some long term lithological or diagenetic control on the remanent magnetism of the sediment that was not effectively normalised by the procedures used. On the other hand, because of its better defined and persistent character in the record, we feel more confident in interpreting the 90-cm wavelength variation in NRM/ARM as a previously undescribed component of secular variation of palaeointensity. Its apparent period is about 43,000 yr.

A bandpass filter centred on this wavelength (Fig. 3) shows that this component has about a 30% variation about its mean value, an amplitude of magnitude similar to that obtained for palaeointensity variation¹³. We note, however, that our interpreted palaeointensity variation has a period about four times greater than previous estimates¹⁴. This apparent discrepancy may be due to an inadequate definition of variations in the relatively short archaeomagnetic record on which previous estimates of palaeointensity periodicity have been made. On the other hand, it may simply reflect a more complicated variation in palaeo-

intensity than previously suspected. The cause of this discrepancy is difficult to resolve, particularly because our record seems to lack sufficient resolution to detect the shorter periodicity of palaeointensity variation (about 10,000 yr) estimated from the archaeomagnetic record.

The long period and relatively large amplitude of the inferred 43,000-yr palaeointensity variation suggest that it is due primarily to a secular variation in the strength of the main dipole field, and consequently must reflect processes within the Earth's core associated with its generation. Malkus¹⁵ has proposed that the geomagnetic dynamo may be driven by forces arising from the precession of the Earth's rotational axis. The efficacy of this energy source in driving the geomagnetic dynamo has been questioned by some^{16,17} while others^{15,18} maintain that it is the basic driving mechanism (although convection may play some part). The Earth's rotational axis precesses about the normal to ecliptic with a period of about 21,000 yr. The angle between the rotational axis and the normal to the ecliptic (the obliquity), however, does not stay constant but varies between approximately 22.1° and 24.5°, with an average periodicity of about 41,000 yr (ref. 19). The precessional force on the core can be expected to vary with the obliquity. If precession has a role in driving the geomagnetic dynamo, some sympathetic variation with obliquity might be expected in the Earth's magnetic field. We suggest that the apparent 54,000-yr variation in palaeointensity recorded in RC10-167 is near to the periodicity of obliquity, and reflects the modulation of a precessionally-driven dynamo by changes in the obliquity.

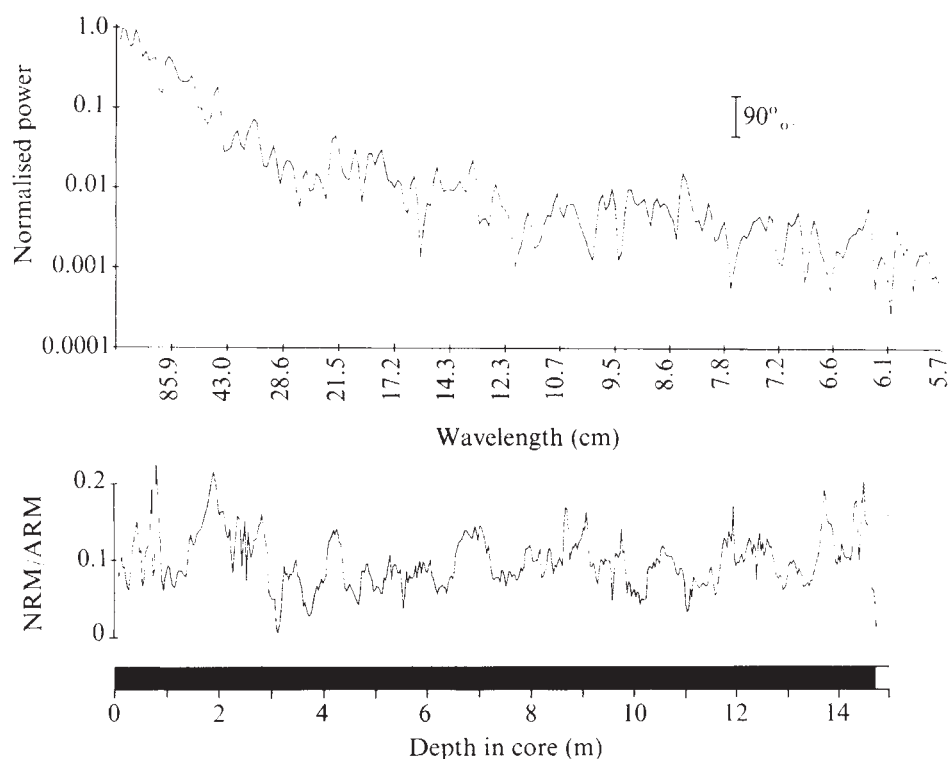


Fig. 2 Lower trace shows the ratio NRM/ARM, against ash-free depth in core RC10-167. The initial set of 448 sample measurements has been interpolated linearly to produce 512 equally-spaced values. Upper trace shows power spectrum of NRM/ARM variation record shown in lower trace, using the fast Fourier transform with a cosine-rectangular window. Estimates of power spectra were smoothed further by a three-point weighted (Hanning) filter. The solid bar is an estimate for the 90% confidence interval.

Although the magnitude of precessional force may vary by only less than 10% between times of maximum and minimum obliquity²⁰, much less than the variation in palaeointensity observed here, the relationship between the magnitude of precessional force and the field generated may not be simple. It is possible that some critical amount of energy is required to sustain dynamo action, as found in laboratory dynamo models²¹. Near this critical value, relatively small variations in contributing forces, such as precession, may have pronounced effects in the magnitude

of the generated magnetic field. Figure 3 shows the variation with time in the Earth's obliquity¹⁹ and in inferred geomagnetic palaeointensity as recorded in core RC10-167, assuming a linear time scale between the top of the core and the level of the Brunhes-Matuyama boundary. There seems to be a good correspondence in the expected sense between the palaeointensity and obliquity variations over this time, with obliquity maxima apparently preceding the inferred intensity maxima by about 5,000 yr.

There is no statistical compulsion to accept at the 90%

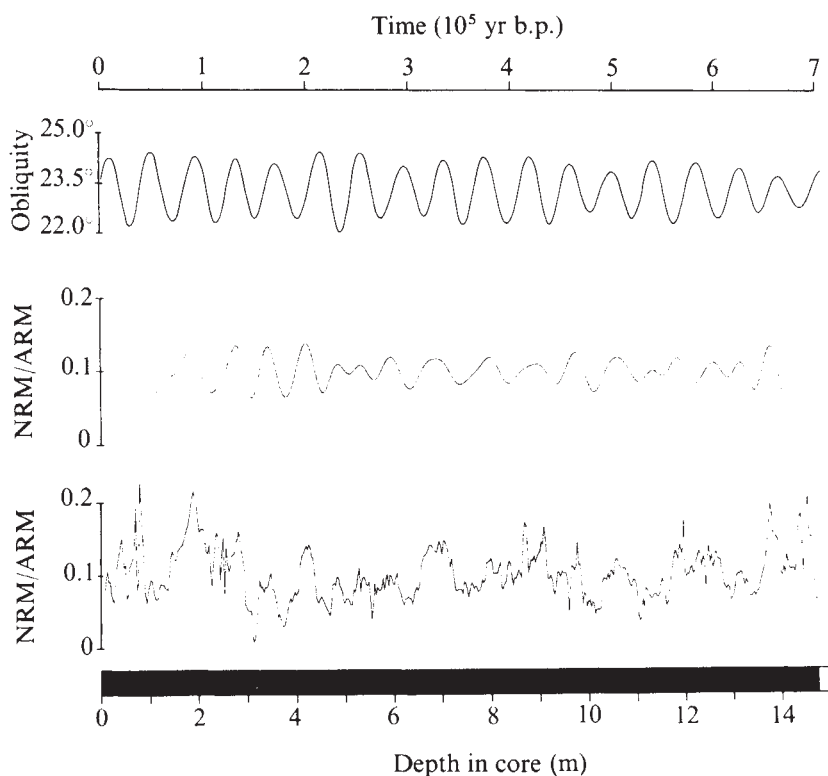


Fig. 3 The variation of NRM/ARM in core RC10-167 (bottom) and the same record (middle) filtered with a cosine-tapered band-pass centred at a wavelength of 90 cm. The time scale is based on the assumption of a uniform deposition rate (volcanic ash layers excluded) between the top of the core ($t = 0$ yr) and the level of the Brunhes-Matuyama magnetic reversal boundary ($t = 700,000$ yr). Also shown (top) is the variation with time in the Earth's obliquity from ref. 19.

confidence level the existence of the 43,000-yr period in the NRM/ARM record on the basis of its power spectral density alone. We have some confidence in its reality, however, on the strength of two arguments—the apparent phase coherence with the variation in the Earth's obliquity and the knowledge that such a correlation is not physically absurd. We recognise, however, that results from a single sediment core should be viewed with caution, especially when a global phenomenon is invoked in explanation. It is possible that the inferred palaeointensity variation in this core reflects a more local palaeomagnetic field behaviour or a physical property of the sediment, in spite of the magnitude and internal consistency of the observed NRM/ARM variation. Other periodicities of palaeofield intensity may also be involved. These may be difficult to identify with certainty in this core because of the limited record length available to characterise any longer period variations, and because of the restriction imposed by the sampling interval and sediment deposition rate in resolving with confidence any shorter period variations. Nevertheless, we feel that the data described here provide sufficient basis for serious consideration of a 43,000-yr variation in the palaeomagnetic field intensity during the past 700,000 yr. Confirmation of the existence of such a variation is being sought in a deep-sea sediment core of different lithology and geographically remote from RC10-167. We point out, however, that of approximately 1,000 cores that we have studied palaeomagnetically from the L-DGO core collection, RC10-167, is unique in having the longest complete record of the Brunhes normal magnetic epoch.

If the apparent correlation between variation in geomagnetic palaeointensity and the Earth's obliquity is substantiated by further work, several implications are apparent. First, it would provide evidence that forces arising from the Earth's precession contribute to driving the geomagnetic dynamo. Second, there is now good evidence that long term climatic variations have been controlled, at least in part, by the Earth's orbital parameters²². Therefore, a correspondence between some component of climatic change and variations in the Earth's magnetic field can be expected, due to a possible common mechanism.

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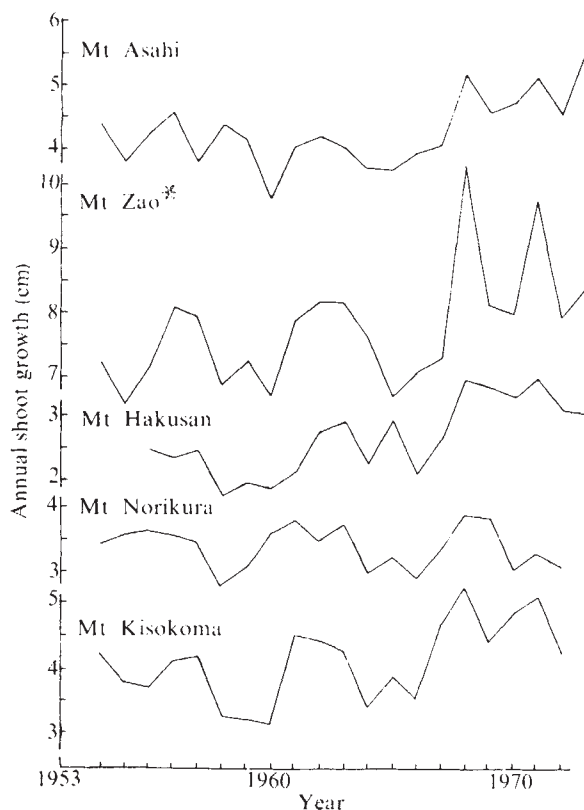
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Growth of *Pinus pumila* and climate fluctuation in Japan

TREE-RING analysis shows a relationship between annual ring growth and climatic changes in the United States¹⁻³ and in the Scandinavian countries⁴. We have measured annual growth in terms of elongation of the terminal shoot of dwarf pine, *Pinus pumila* (Pallas) Regel, which is common in the alpine zone of Japan and eastern Siberia. This tree was used as the indications of annual growth are clearer than in other plants. The ages of branches used for measurement are 50–200 yr, although dating at >100 yr is difficult. We discuss here the relationship between the fluctuation in annual elongation of dwarf pine and climatic changes in Japan or in higher latitude of Northern Hemisphere.

Elongation of the terminal bud of *Pinus pumila* starts in early June and terminates in late July. The buds do not elongate further in the following years. Thus annual elongations of the past years are measurable and the number of years so indicated agrees with the number of annual rings at the node. Annual elongation of the main shoot of the pine was measured on trees in many locations of the alpine zone of Japan. There was uniformity of annual variations in elongation among most of populations of different locations (Figs 1 and 2). Such a uniformity was even observed between the populations of Mt Hakusan and Mt

Fig. 1 Annual shoot growth of *Pinus pumila* in five stands of the alpine zone of Japan. Values for shoot growth are means of 60–20 plants. The stands are shown in Fig. 2. Correlation coefficient between each two stands: Mt Kisokoma–Mt Norikura, $r = 0.396$; Mt Kisokoma–Mt Hakusan, $r = 0.873$; Mt Kisokoma–Mt Zao, $r = 0.785$; Mt Kisokoma–Mt Asahi, $r = 0.701$; Mt Norikura–Mt Hakusan, $r = 0.362$; Mt Norikura–Mt Zao, $r = 0.325$; Mt Norikura–Mt Asahi, $r = 0.072$; Mt Hakusan–Mt Zao, $r = 0.733$; Mt Hakusan–Mt Asahi, $r = 0.674$; Mt Zao–Mt Asahi, $r = 0.768$. *Hybrid population of *P. pumila* and *P. parviflora*.



Asahi, though these two mountains are about 950 km apart. This result suggests that the growth of the pine is affected by climatic factors that predominate over a wide region of Japan.

Annual elongation of the pine of Mt Kisokoma correlated positively with the total sunshine in June, July and August of the previous year, and/or with the mean temperature of June, July and August of the previous year (Fig. 3). There is presumably some dependence of growth on rain or snowfall. Correlation between growth and sunshine, however, is apparent even if effects of rainfall are ignored.

There are many reports on climatic changes of the Earth⁵⁻⁷. We measured the annual elongation of the old

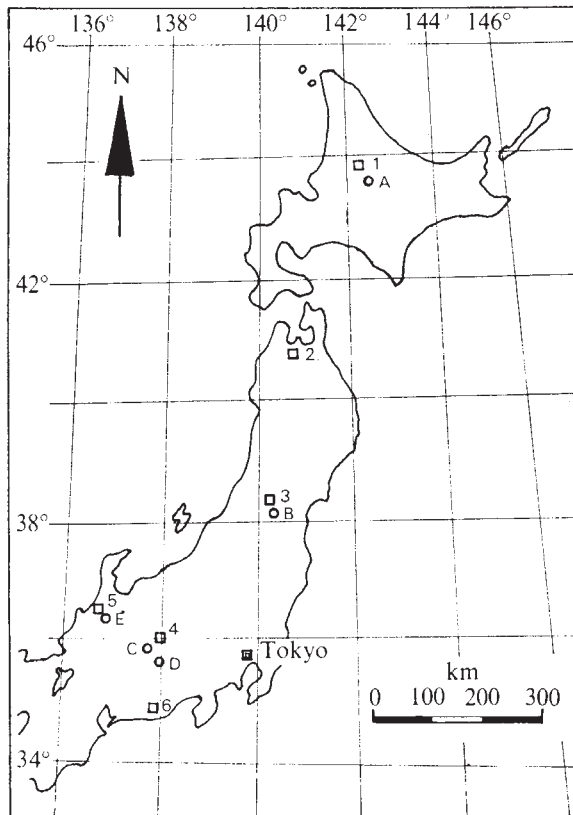


Fig. 2 The location of the sample stands of *P. pumila* and the meteorological stations where climatic data was collected. A, Mt Asahi; B, Mt Zao; C, Mt Norikura; D, Mt Kisokoma; E, Mt Hakusan; 1, Asahikawa; 2, Aomori; 3, Yamagata; 4, Matsumoto; 5, Kanazawa and 6, Hamamatsu.

pine (about 200 yr old) at Mt Norikura going back to 1890, and its fluctuation was compared with the data on fluctuation of temperature around the world shown by G. S. Callendar (Fig. 4). In Fig. 4, the temperature of sub-arctic zones around 1890–1900 is shown as low and that around 1940–1950 as high; elongation of the shoot was small around 1900–1910, and large around 1940–1950. Thus fluctuation in elongation varies with temperature in the sub-arctic (60–73 °N) with a slight time lag. This coincidence with temperature fluctuation in the sub-arctic zone rather than in Japan is of note. A similar relationship was seen in other population of *Pinus pumila* with other data of temperature fluctuation in the Northern Hemisphere (data of J. M. Mitchell). Thus the growth of the pine in the alpine zone of Japan accurately reflects the fluctuation of temperature in the Northern Hemisphere, especially in the sub-arctic zone.

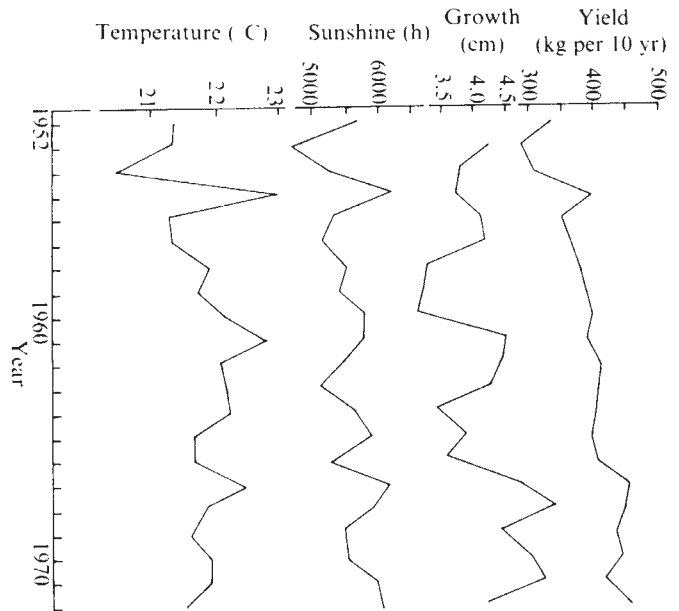


Fig. 3 Fluctuation of annual shoot growth of *Pinus pumila* of Mt Kisokoma in Japan and fluctuation of temperature (mean of June, July and August at six stations) and sunshine (sum of June, July and August at six stations) and mean rice yield per acre in Japan^{8,9}. Each value of shoot growth is the mean of 40 plants. The six meteorological stations are shown in Fig. 2. Correlation and regression coefficients: (1) Temperature of previous year (x)—shoot growth (y), $r = 0.291$, $Y(\text{cm}) = 0.303x(^{\circ}\text{C}) + 2.538$. (2) Sunshine of previous year (x)—shoot growth (y), $r = 0.407$, $Y(\text{cm}) = 0.001x(\text{h}) + 0.726$. (3) Rice yield previous year (x)—shoot growth (y), $r = 0.518$, $Y(\text{cm}) = 0.007x(\text{kg per 10 a}) + 1.306$. (4) Sunshine (x)—temperature (y), $r = 0.636$, $Y(^{\circ}\text{C}) = 0.001x(\text{h}) + 17.080$. (5) Sunshine (x)—rice yield (y), $r = 0.685$, $Y(\text{kg per 10 a}) = 0.072x(\text{h}) - 7.714$. (6) Temperature (x)—rice yield (y), $r = 0.552$, $Y(\text{kg per 10 a}) = 43.207x(^{\circ}\text{C}) - 549.355$.

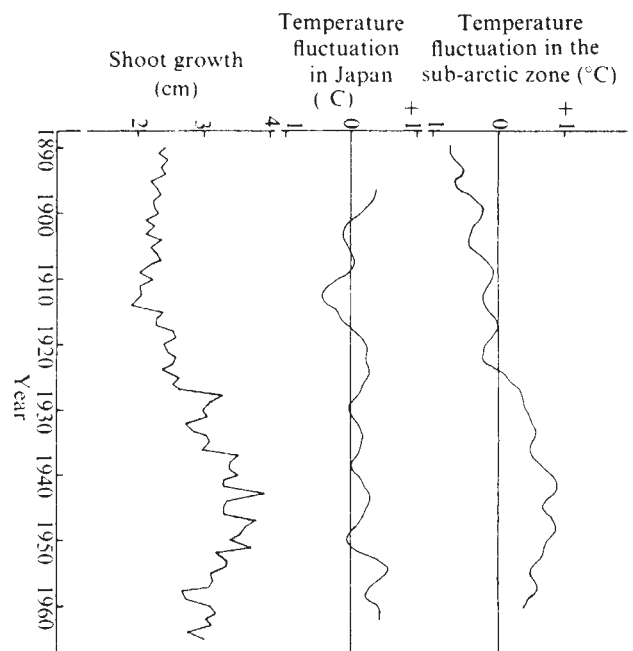


Fig. 4 Fluctuation of annual shoot growth of *Pinus pumila* of Mt Norikura in Japan and fluctuation of temperature in Japan and the sub-arctic (60–73 °N) zone. Values of shoot growth are the means of 33 plants. Climatic data from G. S. Callendar⁵. Correlation coefficient and regression equations: (1) Temperature in the sub-arctic zone (x)—pine growth in Mt Norikura (y), $r = 0.835$, $Y(\text{cm}) = 0.895x(^{\circ}\text{C}) - 2.616$. (2) Temperature in Japan (x)—pine growth in Mt Norikura (y), $r = 0.396$, $Y(\text{cm}) = 0.982x(^{\circ}\text{C}) + 2.687$. (3) Temperature in the sub-arctic zone (x)—temperature in Japan (y), $r = 0.298$, $Y(^{\circ}\text{C}) = 0.144x(^{\circ}\text{C}) + 0.048$.

Annual fluctuation of the pine elongation in Mt Kisokoma was also compared with annual fluctuation of the mean yield per acre of rice in Japan (Fig. 3). Rice yield per acre in Japan is affected mainly by sunshine and temperature of June, July and August. A similar trend of fluctuation is seen between pine growth and rice yield per acre of the previous year.

Study of *Pinus pumila*, may thus be useful in the estimation of past environmental changes such as fluctuations of temperature and sunshine.

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Strong attenuation of Rayleigh waves in Tibet

THE Tibetan Plateau, which has an average elevation of 5.0 km over 7×10^5 km², is the largest topographic mass above sea-level on the Earth. Because it is covered with Cretaceous limestones¹ its uplift must be Tertiary, and is probably related to the Himalayan continental collision. There is considerable debate concerning its present structure and mode of formation. Various authors have theorised that Tibet was uplifted by underthrusting of a second crustal layer^{2–6}; by horizontal compression and thickening^{7–9}, or by low-density material in the mantle¹⁰. All that is known with certainty is that it is isostatically compensated¹¹, and covered with widespread Neogene calc-alkaline volcanic rocks¹². The study of Rayleigh wave attenuation reported here indicates that the lowermost part of the crust is partially molten, and that uplift has been due to horizontal compression.

Because of its size, uniformity and difficult access, Tibet is most easily studied using seismic surface waves. Rayleigh waves have the advantage that they are not confused with Love waves even if horizontal refraction causes the waves to arrive from an unknown direction. Because there are no long-period stations north of Tibet, however, the attenuation cannot be measured by using two stations on one raypath. Instead, we have compared Tibetan paths with other reference paths that do not cross the plateau (Fig. 1). By normalising the wave spectra at short periods, the azimuthal factor in the source spectrum can be cancelled approximately, and the spectral differences at longer periods can be ascribed to attenuation or scattering. We chose a period of 20 s for normalisation, because it is the average spectral peak on the seven reference seismograms. This method gives only a minimum value for attenuation, but records from the nuclear explosion of October 14, 1970 at Lop Nor give similar results without any normalisation.

Data from six earthquakes (yielding 14 Tibetan paths) are shown in Fig. 2. They were obtained by digitising the long-period vertical records from WSSN stations at Kabul, Nilore, Lahore, New Delhi, Shillong and Hong Kong. Before

differencing, the raw spectra $A(\omega)$ were smoothed by the operation:

$$\bar{A}(\omega) = \frac{\int_0^\infty A(\xi) \exp\left(-50 \left(\frac{\xi - \omega}{\omega}\right)^2\right) d\xi}{\int_0^\infty \exp\left(-50 \left(\frac{\xi - \omega}{\omega}\right)^2\right) d\xi}$$

On 11 of the 14 paths across Tibet, a large reduction in the amplitude of long period (40–50 s) Rayleigh waves is evident. This cannot be due to differences in the instruments (they are identical) or in the source spectrum or path length. They must result from different velocity structures under the stations, scattering or attenuation in Tibet.

The effect of the first is probably minimal, because all stations except Kabul are on stable continental crust of about 40-km thickness^{13,14}. Selective reflection of long periods at the boundaries of Tibet is probably more important. The plateau is,

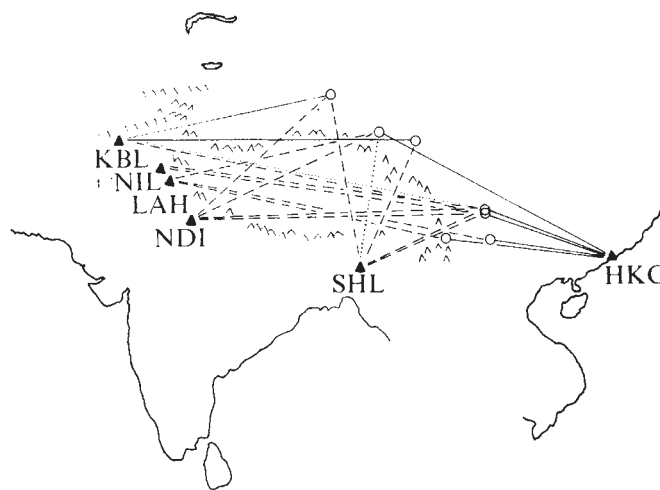


Fig. 1 Surface wave paths used to study attenuation. The Tibetan Plateau is outlined with mountain symbols; ○, earthquakes; ▲, stations; ---, $Q_R(40) < Q_R(20)$; ·····, $Q_R(40) > Q_R(20)$; ———, reference paths.

however, bounded by mountain ranges (Himalayas and Kun Lun) in which the crustal thickness change is spread out over more than one wavelength. For such dipping interface problems, it has been found that reflection of Love surface waves is very small¹⁵, so we do not believe that this effect accounts for the large spectral differences. These have been interpreted as the result of attenuation, so that:

$$\min(Q_R^{-1}) = \frac{PG}{\pi\Delta} \ln \left[\frac{\bar{A}(\omega) \bar{A}_R(0.05)}{\bar{A}_R(\omega) \bar{A}(0.05)} \right]$$

where Q_R is the Rayleigh elastic quality factor, P is period, G group velocity, Δ the path length, and $\bar{A}_R(\omega)$ the spectrum on the reference path.

The minimum attenuation obtained (Fig. 3) has a peak at 50-s period. The half-width of the peak is a factor of 2.8 in frequency, which is too narrow to be produced by frequency dependence of a geologically complex medium with various relaxation times, but is more likely to result from an increase of attenuation with depth, so that longer-period waves are more

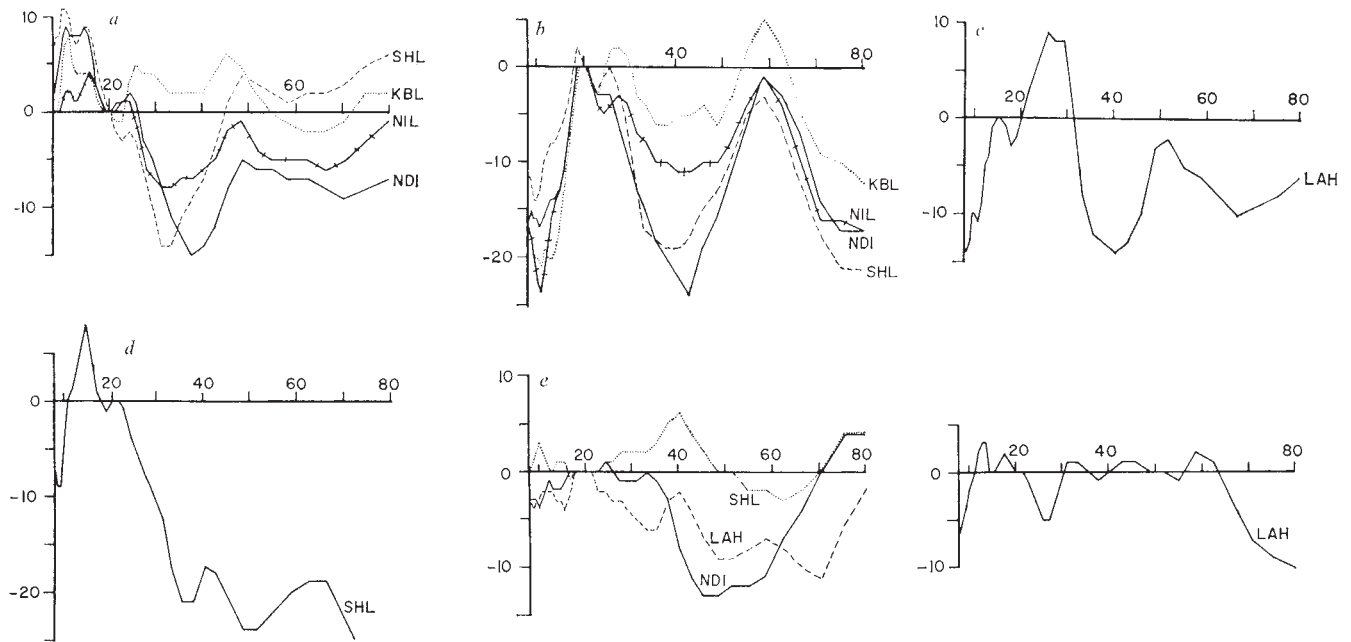


Fig. 2 Power spectrum differences in dB between Rayleigh waves crossing Tibet and those on reference paths from the same source. Period in s is given on the horizontal scale. The difference is set to zero at 20 s to normalise each curve. *a*, August 16, 1971 (1854 h), ref. HKC. *b*, August 16, 1971 (2237 h), ref. HKC. *c*, February 5, 1966, ref. HKC. *d*, March 24, 1971, ref. KBL. *e*, March 16, 1964, ref. HKC. *f*, September 28, 1966, ref. HKC.

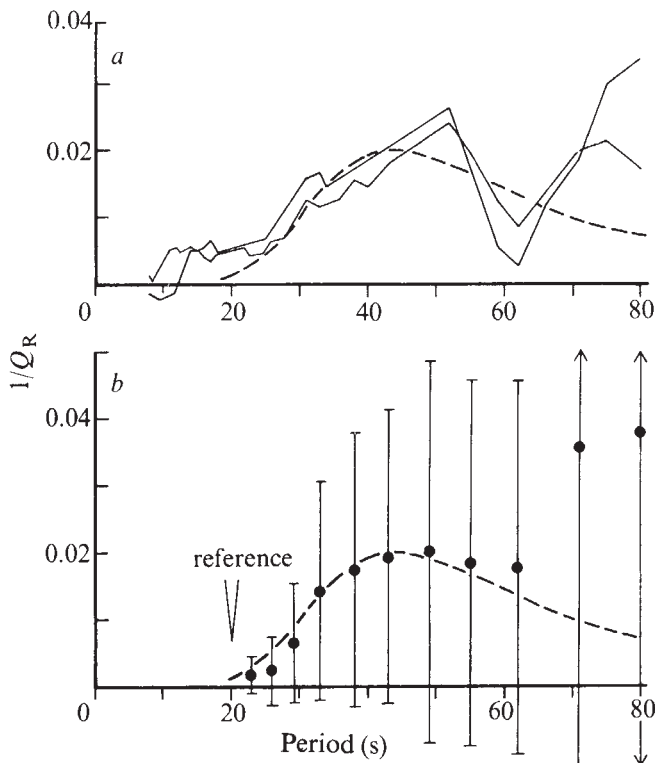


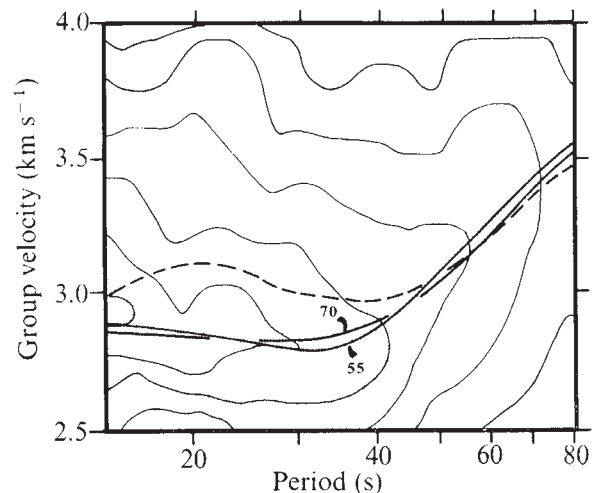
Fig. 3 Attenuation of Rayleigh waves determined from (a) nuclear test data and (b) all data combined. Bars show the s.d. in attenuation. -----, Single attenuating layer at 70-km depth. Attenuation goes to zero at 20-s period because of the normalisation performed.

strongly affected. Assuming also that there are no purely dilatational dissipations, we can find the depth to the centre of the attenuating layer. Using an Earth velocity model compatible with observations on the group velocities of Rayleigh waves and the equations for a horizontally-layered Earth¹⁶, we find that the best-fitting single attenuating layer would be centred at 70 km below the surface. Its 'attenuation thickness' (ratio of layer

thickness to Q^{-1} for shear waves) is 2 km, that is, if the layer is 10 km thick, it has a Q of 5. Such extremely low values of Q are only found in two-phase (partially melted) rocks at such high pressures. There is, also, a suggestion of a second, deeper attenuating layer in the data (Fig. 3), which is not discussed here because it is so poorly resolved.

The geological significance of the inferred partially-molten layer depends on whether it is in the crust or the mantle. From the size of the gravity anomaly¹¹ we can estimate that the crust is between 55 km thick (if the mantle is as hot and light as it is beneath mid-ocean ridges) and 70 km thick (assuming a normal mantle¹⁷). Either of these thicknesses is compatible with the group velocity data (Fig. 4) because the lower crustal shear velocity is unknown. The velocity required in the 70-km model

Fig. 4 Rayleigh wave group velocity data from 20 paths consisting of at least 50% Tibetan Plateau. Records were analysed by spectral amplitude technique¹⁸ and the spectral amplitudes have been summed. Total power is contoured in 5-dB intervals, and the shaded area indicates a swath within 2 dB of the local maximum. —, Models with crustal thicknesses of 55 and 70 km. -----, Model with two normal continental crusts superposed.



(3.8 km s⁻¹) is more plausible, however, than the very low velocity required in the 55-km model (3.5 km s⁻¹). Also, the volcanics at the surface are clearly of crustal origin⁸, and it is natural to associate the attenuating layer with their source region.

We conclude that Tibet has a crust of about 70-km thickness, of which the lowermost part is partially molten. The inferred high temperature of the Moho is inconsistent with the crustal-underthrusting model for the formation of Tibet⁹. It is suggested that the high temperatures may predate the Himalayan continental collision, and that they may have been responsible for extensive horizontal compression in Tibet after that collision, thus explaining the anomalously thick crust. At present, Tibet overlies an asthenosphere which extends into the lower crust, precluding any long term mechanical strength. This weakness at shallow depths is responsible for the uniformity of Tibetan elevations over wide areas in the centre of an orogenic zone.

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mantle 'convection' cells and associated orogenic belts is similar to that of the mobile belts, but dwarfs the dimensions and periodicity of the Archaean greenstone belts. Experiments^{5,6} show that convection leads to the formation of bimodal structural patterns; where convection cells may be primary or secondary, each having contrasting dimensions and orientations. I propose here that mobile belts represent 'super-greenstone belt' structures due to primary linear asthenospheric upwellings, whereas secondary upwellings (Richter rolls) give rise to greenstone belts, usually at a high angle to the trend of the primary structure. The initial primary upwelling divides the pre-greenstone terrain crust into two portions, separated by a zone which undergoes greenstone belt evolution that outstrips that of the relatively sluggish belts within the adjacent cratons (Fig. 1). This contrast in evolution rate and scale between mobile belts and greenstone terrain is due to respective differences in heat transfer from the underlying asthenosphere.

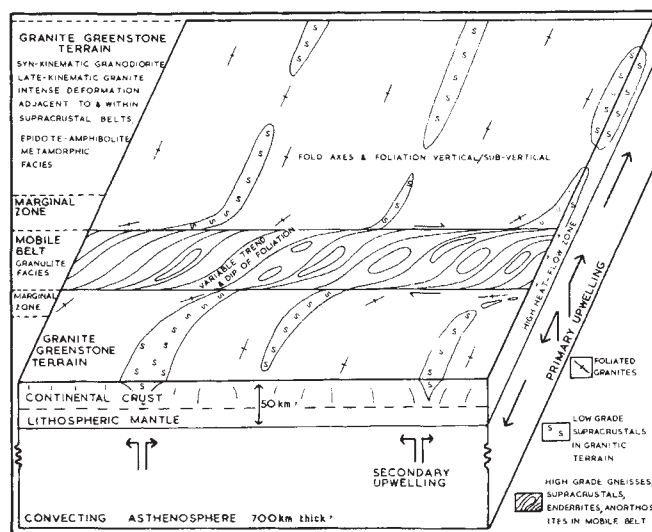


Fig. 1 Oblique view and section of an idealised granite-greenstone-mobile belt relationship.

African Archaean mobile belts and granite-greenstone terrain

ARCHAean granite-greenstone terrains such as the west African and Rhodesian Cratons, are frequently bordered by pene-contemporaneous, long-lived, high-grade mobile belts containing granulite facies supracrustals, enderbites, granite gneisses and anorthositic. For example, in Sierra Leone¹, the granite-greenstone terrain contains low-grade supracrustal belts along NNE-SSW trends, while the discordant Kasila Group granulite facies belt trends NW-SE, and forms the coastal border of the West African Craton. A similar relationship is found between the Rhodesian and Kaapvaal Cratons, which are separated by the east-west trending Limpopo Belt^{2,3}. The metamorphic, structural and lithological contrasts between mobile belts and granite-greenstone terrain have been ascribed to contrasts in heat-flow regime. I attempt here to correlate field and experimental data to elucidate Archaean mobile belt-craton relationships.

Greenstone belts were probably initiated along linear 'rift-type' structural weaknesses which developed as a consequence of tensional forces within the lithosphere at right angles to their trend⁴. These forces could be caused by drag from an underlying upwelling asthenosphere, as for example, in present-day axial rift zones in continental swells and mid-ocean ridges. The scale of contemporary

Heat production and transfer within the Archaean asthenosphere and mesosphere was probably more than twice its present value⁵, and may have involved turbulent, rather than steady-state convective cells⁶. Elder⁷ has also shown that within a convecting system there is a critical lateral distance between the mobile belt and the development of secondary linear systems. In the African Archaean cratons the greenstone belts associated with the secondary cells are frequently enveloped by the deforming marginal zones of the mobile belt⁴ indicating that this critical distance was small. This distance is governed by the loss of heat from the upper surface of the primary cell, which is the asthenosphere-lithosphere boundary, and the thickness of the convecting asthenosphere layer. Highly efficient loss of heat to the lithosphere could have arisen by convection within its granitic layer⁷, since convective diapirs of granite frequently distort the originally linear trend of the secondary cells to produce the less ordered structure typified by the 'gregarious batholith'. Elder also noticed that natural breaks occurred within the secondary cells, which could explain the longitudinal impersistency of some supracrustal belts.

The granulite facies mobile belts are characterised by linear and dome-basin tectonic styles, where extreme deformation in the linear portions limited grain growth, and produced fine-grained basic granulites and platy quartz-feldspathic rocks. The relative movement of the bordering cratons with respect to the mobile belt, by pure

and/or simple shear, is responsible for the plastic deformation in belts and the development of thrusts at their margins. The cause of the movement may be due to small angular shifts in the orientation of the bordering cratons, as they succumb to the eddy currents in the underlying turbulently convecting asthenosphere.

The high heat flow in the mobile belt may also be the key to the close spatial and temporal relationships between granulite facies rocks and layered calcic anorthosites⁴. Anorthosites occur within and adjacent to mobile belts, and are characteristically coarse-grained, even pegmatitic, suggesting intrusion of a hydrous magma into hot crust. The restricted occurrence of layered calcic anorthosites within the Archaean⁴, and of most massif-type anorthosites to mobile belts of Proterozoic age, suggests that they are formed within zones of extremely high heat flow. Phanerozoic anorthosites are also restricted to zones of high heat flow where lithospheric plates have⁸ or are separating⁹.

Ensialic Proterozoic belts¹⁰, and those of the Pan-African, may be caused by processes similar to those of Archaean age. The post-Archaean lithosphere, however, was relatively thicker, stronger and more sedate¹¹, failing to allow the formation of secondary structures, so that greenstone belts did not form contemporaneously with mobile belt activity. Phanerozoic 'plate tectonics' may represent the final examples of a decaying process now only capable of producing endosialic orogens on the margins of continental lithosphere resistant to mobile belt formation.

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Isolation of amitrole-degrading bacteria

THE herbicide, amitrole (3-amino-1,2,4-triazole) is degraded in soil within a matter of weeks under favourable conditions¹⁻⁴. This degradation may be microbiological since it is negligible in soils sterilised by steam^{1-3,5} or chemicals^{2,3}. Kaufman *et al.*⁶, however, consider amitrole breakdown to be primarily due to chemical processes since amitrole-degrading organisms have never been isolated from soil. They found that degradation of amitrole continued in chemically sterilised, though not in autoclaved soil. We report here the isolation of amitrole-degrading micro-organisms from soil, thus strengthening the case for microbial degradation.

Tenfold serial dilutions (from 10^{-2} to 10^{-8}) of a sandy clay loam soil were prepared in each of three media: basal medium (0.03 g K_2HPO_4 , 0.01 g $MgSO_4 \cdot 7H_2O$ and 30 p.p.m. amitrole, pH 7.3), medium 2 (basal medium plus 3% sucrose) and medium 3 (basal medium plus 3% sucrose, 50 p.p.m. $(NH_4)_2SO_4$, 100 p.p.m. peptone and 20 p.p.m. yeast extract). The test-tubes of inoculated media were incubated at 25 °C over 6 weeks and the amitrole content in those with visible growth was measured by Sund's method⁷. Amitrole disappeared completely in medium 2 at soil dilu-

tions of 10^{-3} to 10^{-6} (Table 1), but no degradation could be detected in any other treatment.

Pure cultures were isolated from tubes showing degradation by dilution plating on to the same medium solidified with 1.5% agar. Of 36 representative isolates reinoculated into medium 2, 10 were capable of degrading amitrole; nine of these were Gram-positive rods (*Bacillus* spp. and *Corynebacterium* spp.) and one was identified as *Pseudomonas* sp.

Table 1 Degradation of amitrole by soil samples

Soil dilution	Incubation time (weeks)					
	1	2	3	4	5	6
10^{-2}	—	—	—	—	—	—
10^{-3}	—	—	+	+	+	+
10^{-4}	—	—	—	+	+	+
10^{-5}	—	—	—	—	—	+
10^{-6}	—	—	—	—	—	+
10^{-7}	—	—	—	—	—	—
10^{-8}	—	—	—	—	—	—

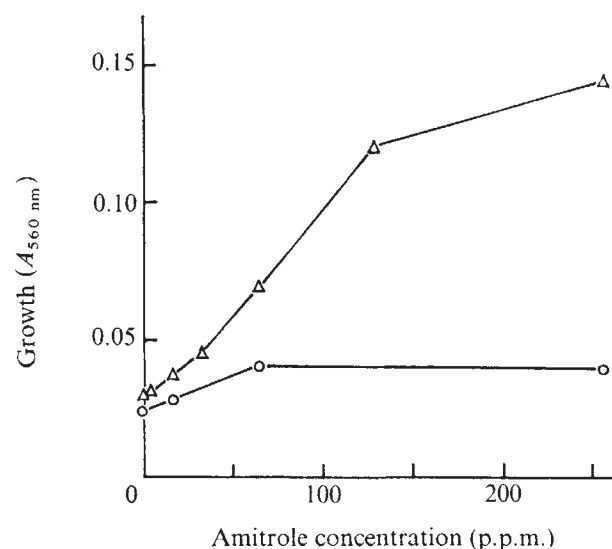
Diluted soil samples were incubated in 10-ml aliquots of medium 2 at 25 °C; —, no degradation; +, degradation.

The relationship between growth of one amitrole-degrading isolate and amitrole concentration was determined by growing the isolate in Erlenmeyer flasks of basal medium or medium 2 containing various amitrole concentrations. To prevent aerial contamination of the medium with combined nitrogen, each flask was sealed with a rubber stopper and connected with the exterior only by a U-tube containing H_2SO_4 . In these conditions the isolate was unable to fix molecular nitrogen.

Growth was roughly proportional to herbicide concentration up to 256 p.p.m. amitrole in the medium with sucrose (Fig. 1). There was little growth in basal medium with amitrole as the sole source of organic carbon as well as nitrogen.

The inability of other workers to isolate actively degrading organisms may have been due to their use of enrichment media with amitrole as sole source of organic carbon rather than nitrogen. It also seems that the decomposition of amitrole is inhibited in soil dilutions less than 1 in 1,000. This could be due to preferential use of other nitrogen

Fig. 1 Effect of amitrole concentration in the medium on growth of isolate Q₅ after incubation for 17 d at room temperature (20 °C average $\pm$ 6 °C). Mean of three replicates, in basal medium (○) and medium 2 (△).



sources present in soil, since the presence of combined nitrogen in medium 3 prevented degradation of amitrole at any soil dilution.

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Frequency of heavy-metal resistance in bacteria from inpatients in Japan

In many bacteria, resistance to heavy metals is associated with a plasmid¹⁻⁴. R plasmids in *Escherichia coli* can determine resistance to several metallic ions such as mercury, cobalt and nickel³, and a penicillinase plasmid mediating resistance to mercury, cadmium, arsenate, arsenite, lead and zinc has been observed in *Staphylococcus aureus*^{1,2}. Mechanisms controlling bacterial resistances to mercury and cadmium are quite different although both are mediated by the same penicillinase plasmid⁴. Furthermore, microorganisms generally detoxify mercurial compounds metabolically by the formation of volatile mercury⁵⁻⁹ or mercury mercaptides¹⁰⁻¹². It is of interest that resistance to these heavy metals is mediated by the plasmids which determine resistance to antibiotics. Most of these heavy metals are established or possible causes of environmental pollution; methyl mercury causes 'Minamata disease'^{13,14} and cadmium causes 'Itai-Itai disease'^{15,16} in Japan. The role of R plasmids in drug resistance has been widely studied¹⁷⁻²¹, and extrachromosomal determinants are a main cause of the increase in numbers of drug-resistant bacteria. Studies of heavy metal resistance have attempted to establish a relationship between resistance to heavy metals and to drugs in the hospital environment^{22,23}. The factors selecting for these heavy-

metal-resistant bacteria have not yet been identified. We believe that heavy-metal-resistant microorganisms do not arise by chance, but, that there must be selectional factors beyond mere drug resistance. One of these selectional factors may be environmental contamination by heavy metals. To investigate this possibility, we studied the frequency of drug and heavy-metal resistance in clinical isolates of *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *S. aureus*. We report here that the frequency of heavy-metal resistance in these strains was the same as, or higher than that of antibiotic resistance. Some R plasmids in *E. coli* and *K. pneumoniae* also carried a resistance to Hg.

Figure 1 shows the distribution curves of Hg, Cd, As and Pb resistances (from agar dilution tests), in 564 strains of *E. coli*, 331 strains of *K. pneumoniae*, 787 strains of *P. aeruginosa* and 515 strains of *S. aureus*. The distribution of susceptibility to most of these heavy metals, against which a resistance developed, is bimodal. But in the case of a Cd resistance in *S. aureus*, there were three groups of Cd sensitivity.

The frequency of a resistance to these heavy metals and to several drugs for the tested strains is shown in Table 1. The frequency of the heavy-metal-resistant strains was the same as, or higher than the frequency of drug-resistant strains. Many strains were resistant to heavy metals, but remained sensitive to the antibiotics (21.6% of *E. coli*, 27.2% of *K. pneumoniae*, 19.5% of *P. aeruginosa* and 8.0% of *S. aureus*).

We selected 348 strains of *E. coli* and *K. pneumoniae* resistant to Hg and examined the conjugal transferability of their resistance by using two recipients. One recipient, *E. coli* K12 ML1410-Nx, a nalidixic acid-resistant mutant and an As-sensitive segregant of *E. coli* K12, was used for the transfer of R plasmids in the first cycle. The other recipient, *E. coli* JE17-Rif, isolated in our laboratory, is a rifampicin-resistant mutant and was used for the second cycle of transconjugation. Both recipients are sensitive to Hg, As, streptomycin (SM), tetracycline (TC), chloramphenicol (CM), kanamycin (KM) and gentamicin (GM) but resistant to Cd. Among 231 strains of Hg-resistant *E. coli*, 207 Hg-resistant R plasmids were isolated, and of these 195 were As resistant. Among 117 Hg-resistant *K. pneumoniae*, 110 R plasmids carrying an Hg resistance were also observed: 102 of these 110 R plasmids carried resistance to As. In *E. coli*, R plasmids with a resistance to R(Hg,As; SM,TC,CP,KM) were isolated most frequently, followed by R(Hg,As, SM,TC,CP), R(Hg,As, SM,KM), R(Hg,As, TC,CP) and R(Hg,As, SM,TC,KM), in that order. A similar result was obtained in *K. pneumoniae*. The resistance patterns of R plasmids were similar to those of the resistant strains that were frequently isolated, showing that Hg resistance in these enterobacteria is mainly due to the presence of R plasmids (Table 2).

Transferable R plasmids carrying Hg resistance were present in

Fig. 1 The minimum inhibitory concentration (MIC) distribution curves of *a*, Hg; *b*, As; *c*, Cd and *d*, Pb resistances (by agar dilution test) for 564 strains of *E. coli* (●), 331 strains of *K. pneumoniae* (▲), 787 strains of *P. aeruginosa* (■) and 515 strains of *S. aureus* (□). The tested strains were isolated in the Clinical Laboratory of the Jikei Hospital between 1974 and 1976. The metals, Hg, Cd, As and Pb were used as HgCl₂, CdCl₂, Na₂HAsO₄ and Pb(CH₃COO)₂, respectively. Nutrient agar was used for the metal resistance test because HgCl₂ was stable in it. The distribution curves of Hg, Cd and As resistances show the clear-cut distinctions between the 'sensitive' and 'resistant' populations, except that the Cd resistance in *S. aureus* is divided into three groups. In the case of the Pb resistance in *S. aureus*, the distribution pattern clearly revealed two peaks. In *E. coli*, *K. pneumoniae* and *P. aeruginosa*, the distribution curves indicated that the resistance concentrations of each metal were 10 µg ml⁻¹ for Hg, 400 µg ml⁻¹ for Cd and 400 µg ml⁻¹ for As. In *S. aureus*, the resistance concentrations of Hg, As, Pb, high and intermediate Cd were 10 µg ml⁻¹, 400 µg ml⁻¹, 100 µg ml⁻¹ and 10 µg ml⁻¹ respectively.

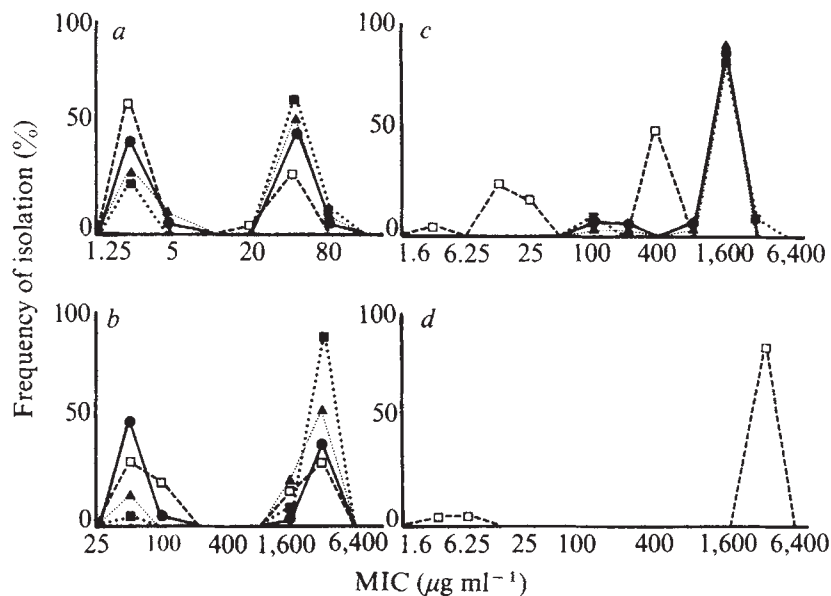


Table 1 Frequency of heavy-metal resistance and drug resistance in *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* clinical isolates

		<i>E. coli</i>	<i>K. pneumoniae</i> No. of resistant strains*	<i>P. aeruginosa</i>	<i>S. aureus</i>
Metal†	Hg	323 (57.3)	218 (65.9)	591 (75.1)	187 (36.3)
	Cd	524 (92.9)	324 (97.9)	759 (96.5)	491‡ (95.3)
	As	342 (60.6)	276 (83.4)	779 (98.9)	251 (48.7)
	Pb	—§	—	—	473 (91.8)
Drug**	PC	ND	ND	ND	454 (88.2)
	SM	377 (66.8)	223 (67.4)	405 (51.5)	144 (28.0)
	TC	344 (61.0)	170 (51.4)	125 (15.9)	171 (33.2)
	CP	315 (55.9)	163 (49.2)	324 (41.2)	113 (21.9)
	KM	186 (33.0)	122 (36.9)	404 (51.3)	119 (23.1)
	EM	ND	ND	ND	220 (42.7)
	JM	ND	ND	ND	198 (38.4)
	GM	9 (1.8)	12 (3.8)	108 (13.7)	0 (0)
Total no. of strains		564	331	787	515

*Figures in parentheses represent % of total isolates.

†Hg: HgCl₂, Cd: CdCl₂, As: Na₂HAsO₄, Pb: Pb(CH₃COO)₂.

‡High Cd: 258 (50.1%), intermediate Cd: 233 (45.2%).

§We cannot detect Pb-resistant strains since the distribution pattern of Pb resistance shows only one peak.

**Streptomycin(SM), tetracycline(TC), chloramphenicol(CP), kanamycin(KM) and gentamicin(GM) were at a final concentration of 12.5 µg ml⁻¹ for the selection of resistant strains in *E. coli*, *K. pneumoniae* and *S. aureus*. In *P. aeruginosa*, cultures not inhibited by 200 µg of SM, TC, CP and KM per ml or 25 µg of GM per ml were regarded as resistant to each of the drugs. In *S. aureus*, resistance concentrations of penicillin(PC), erythromycin(EM) and josamycin(JM) were 1.6 IU ml⁻¹, 25 µg ml⁻¹ and 25 µg ml⁻¹, respectively. ND, not determined.

most (89.6% of *E. coli* and 94.0% of *K. pneumoniae*) of the Hg-resistant isolates. It should be noted that there were three R plasmids carrying only Hg and As resistance in *K. pneumoniae*. These three R plasmids were quite stable in both *E. coli* K12 ML1410-Nx and *E. coli* JE17-Rif (Table 2). This is of interest as mercury is an environmental pollutant.

Thus, frequency of a heavy-metal resistance was the same as, or

higher than, that of an antibiotic resistance, and many isolates were heavy-metal resistant but drug sensitive. We isolated 317 R plasmids with an Hg resistance from Hg-resistant *E. coli* and *K. pneumoniae* (91.1% frequency). In addition, 297 R plasmids mediated As resistance.

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Table 2 Demonstration of R plasmids carrying an Hg resistance in *E. coli* and *K. pneumoniae* isolates and their patterns of resistance to As and antibiotic

Resistance pattern of R plasmid	% of <i>E. coli</i> strains with R plasmid		% of <i>K. pneumoniae</i> strains with R plasmid	
Hg, As; SM, TC, CP, KM, GM	4	4 (1.9)	3	3 (2.7)
Hg, As; SM, TC, CP, KM	65		25	
Hg, As; SM, TC, CP, GM	0	69 (33.3)	3	31 (28.1)
Hg; SM, TC, CP, KM	4		3	
Hg, As; SM, TC, CP	63		24	
Hg, As; SM, TC, KM	9		7	
Hg, As; SM, TC, GM	0	81 (38.2)	2	40 (36.4)
Hg, As; TC, CP, KM	5		4	
Hg; SM, TC, CP	4		3	
Hg, As; SM, TC	8		9	
Hg, As; SM, CP	6		4	
Hg, As; SM, KM	14		10	
Hg, As; TC, CP	12		2	
Hg, As; TC, KM	2	49 (23.7)	1	28 (25.5)
Hg, As; CP, KM	3		0	
Hg; SM, KM	2		2	
Hg; TC, CP	2		0	
Hg, As; SM	2		2	
Hg, As; TC	1		0	
Hg, As; CP	1	4 (1.9)	2	5 (4.6)
Hg, As; KM	0		1	
Hg, As	0	0 (0)	3	3 (2.7)
Total	207		110	

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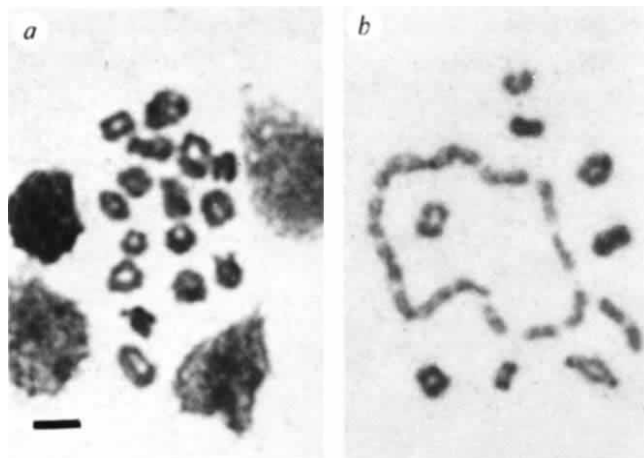
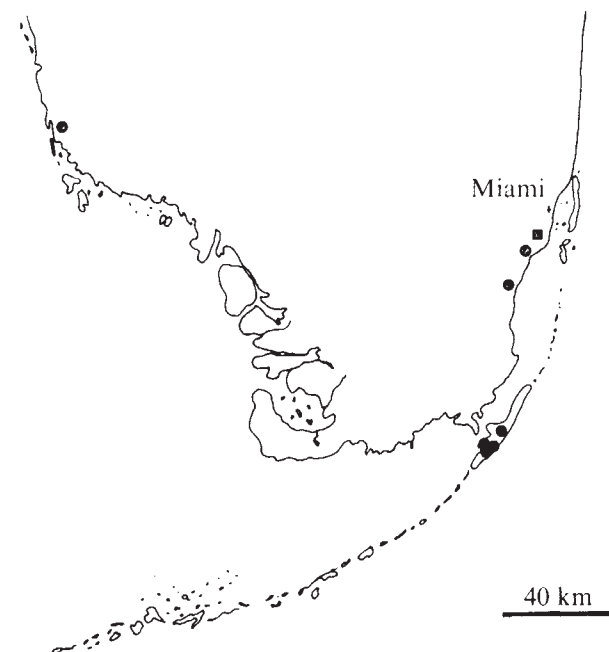


Fig. 1 Meiotic chromosomes in *I. schwarzi*. *a*, female; *b*, male. Whole termite colonies were collected from various locations in southern Florida (Fig. 2), and maintained in the laboratory on a mixture of paper towels and sawdust from termite-infested wood. Chromosome preparations were usually made within a month of collecting the colony. Gonads from winged imagoes were dissected out in 0.45% sodium citrate solution, treated for 3 min with 1.0 N HCl at about 60 °C, transferred to 45% acetic acid for 15-20 min, squashed on a slide under a coverslip, rinsed in distilled water, air-dried, and stained with Giemsa (bar, 5 µm).

have not yet identified the sex chromosomes in this species, this interpretation is supported by the observation that the sex ratio is approximately 1:1 (suggesting chromosomal determination of sex), and by the finding that in the related species *K. approximatus*, where the sex chromosomes can be more easily distinguished morphologically, they are indeed part of the ring. On this interpretation the males of *I. schwarzi* may have, in addition to one 'standard' set of autosomes and an X chromosome, six interchange autosomes and a neo-Y chromosome that evolved from an original standard set and an original Y by a series of reciprocal translocations. Alternate disjunction from the ring of 14 chromosomes formed at meiosis would ensure that the interchange autosomes would always be transmitted to the progeny along with the male-determining neo-Y chromosome (Fig. 3). An alternative possibility is that the

Fig. 2 Locations of colonies of *I. schwarzi* studied in southern Florida. ●, Colonies with 9 bivalents and a ring of 14; ■, colony with 8 bivalents and a ring of 16.



Permanent segmental interchange complex in the termite *Incisitermes schwarzi*

In a cytogenetic survey of the termites of Florida, we have found two species, *Incisitermes schwarzi* (Banks) and *Kaloterms approximatus* (Snyder) (Isoptera; Kalotermitidae), that possess segmental interchange complexes as a regular feature of their breeding system. This report is concerned with the chromosomes of *I. schwarzi*, which has the most extensive complex of reciprocal translocations so far described in any animal species. The complex involves about half of the chromosome set, and is restricted to males. The occurrence of this interchange complex may be related to the social organisation of these insects, to an inbreeding habit of reproduction, or to the evolution of a mechanism of sex determination with multiple sex chromosomes.

The diploid number for both males and females is 32; the chromosome complement contains 4 or 5 acrocentric pairs, the remainder being metacentric or submetacentric. In female meiosis there are 16 bivalents; most of them are ring-shaped, indicating the presence of a terminal chiasma in each chromosome arm (Fig. 1a). In male meiosis in 7 of the 8 colonies studied, there were 9 bivalents plus a ring of 14 chromosomes (Fig. 1b). (The males of one colony had 8 bivalents plus a ring of 16 chromosomes; see Fig. 2.) All of the males from any one colony had the same chromosome configuration. The ring of 14 seems to be composed of metacentrics and submetacentrics; the chiasmata are terminal or nearly terminal. Judging from the chromosome numbers in anaphase I groups and in second meiotic divisions, disjunction from the ring in the first meiotic division seems to be regular, half of the chromosomes moving to one pole, half to the other.

The restriction of the ring to male meiosis indicates that the male is the heterogametic sex, and that the sex chromosomes are incorporated into the ring. Although we

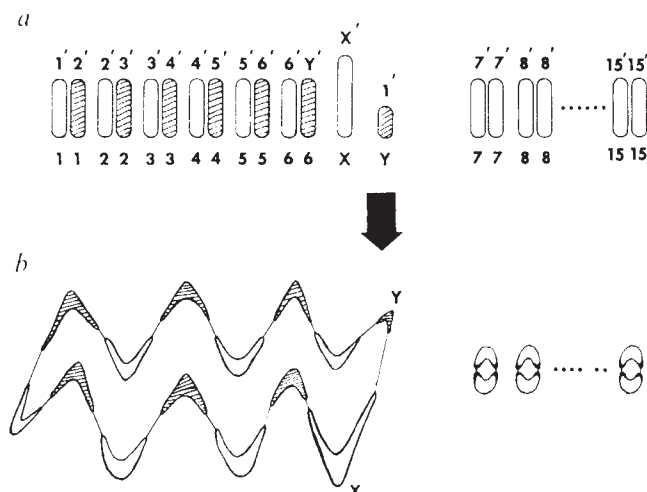


Fig. 3 Diagram illustrating the hypothetical association of a neo-Y and interchange complex (shaded) with the X and the standard set of chromosomes (unshaded) in the male. *a*, Mitotic chromosomes; chromosome ends are numbered to show an example of segmental interchanges that would lead to ring formation in meiosis (*b*). The sizes of the chromosomes are arbitrary; the X and Y chromosomes have not yet been distinguished from the autosomes nor from each other in this species.

whole interchange complex is a set of multiple, male-determining, sex chromosomes.

Such extensive reciprocal translocations, ordinarily expected to lead to chromosome imbalance in gametes and lethality in zygotes, evidently form part of a balanced system that has adaptive value for the species, as in the case of the more extensive exchanges found in the plants *Oenothera* (ref. 1) and *Isotoma* (ref. 2). It is premature to speculate in detail on the genetic and evolutionary significance of the interchange complex in *I. schwarzi*, but the following comments point up some of its implications.

An important genetic consequence of the interchange complex is that its chromosomes, and the standard set with which it is associated, behave as a single linkage group in the male; the interchange complex therefore tends to reduce the genetic variability that would otherwise result from independent assortment of the chromosomes involved. Interchange complexes similar to that found in *I. schwarzi* also occur in the males of the dioecious plant *Viscum* (refs 3, 4), the beetle *Cyrsylus volkameriae* (ref. 5), some populations of the flea *Nosopsyllus fasciatus* (ref. 6), and some species of the centipede *Otocryptops* (refs 7, 8); and possibly in some females of the freshwater copepod *Diaptomus castor* (refs 9, 10). The interchange complex in each of these cases might represent an example of the evolution of a system of multiple sex-determining chromosomes; or it may have arisen by a series of translocations between the Y chromosome of a simpler sex-determining system and the autosomes, as a means of reducing variability, or of maintaining heterozygosity, in a set of autosomal genes. The genetic effect of the interchange complex in all these organisms may be related to the reduction of crossing-over that occurs in the heterogametic sex of other organisms, notably in the males of the fruit fly *Drosophila* and in the females of the silkworm *Bombyx*.

The presence of the interchange complex might be important in the evolution of sociality in these termites, similar in its reduction of genetic variability to the systems of parthenogenesis and male haploidy that characterise the other social insects, the ants, bees and wasps¹¹. The colony might provide a more uniform and stable environment, so that more genetically uniform individuals would be favoured; evolution of genetic uniformity might also be a necessary condition for the coevolution of the intricate hormonal and behavioural interactions that take place among the individual members of the colony. (Note, however, that while *I. schwarzi* and *K. approximatus* both have

extensive interchange complexes, other species of termites—*Termopsis angusticollis* (ref. 12), *Reticulitermes flavipes* (ref. 13), *Odontotermes redemanni* (ref. 14) and *Neotermes jouteli* (unpublished observations)—do not.)

The presence of an interchange complex in *I. schwarzi* may be the evolutionary consequence of inbreeding. The interchange complexes of *Oenothera* and *Isotoma* are associated with a self-pollinating habit of reproduction^{1,2}; Darlington and La Cour¹⁵ obtained evidence in the plant *Campanula* that selfing and inbreeding favour, at least transiently, the maintenance of interchange complexes; and Lewis and John¹⁶ found interchange complexes in some isolated populations of roaches. In termite species in general, the reproductives that appear in the mature colony, at the time of swarming, are usually siblings. Because they are weak fliers and may not travel far from the original colony, and since in any case different colonies in an area may swarm at different times¹⁷, new colonies in general are probably founded by two siblings from the same original colony, rather than by a male and a female from different colonies. These termites therefore may be highly inbred, and thus might provide another example of the correlation between inbreeding on the one hand and the evolution and maintenance of structural heterozygosity on the other.

Note added in proof: We have recently found a third termite species that has an interchange complex in male meiosis: *Neotermes castaneus* (Burmeister) (Kalotermitidae).

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Parallel variation at an enzyme locus in sibling species of field crickets

ELECTROPHORETIC surveys of levels of heterozygosity in animal species have established that populations often contain large amounts of genetic variation at loci coding for soluble proteins^{1–3}. This has led to an extended debate about the causes of such variation. In particular, two opposing views have emerged, one emphasising the importance of balancing selection in maintaining the protein variation (selectionist) and the other emphasising the importance of stochastic processes (neutralist)^{4–6}. Here I examine spatial patterns of variation at a single enzyme locus in two closely related insect species. I argue that since these sympatric species not only have similar mean frequencies of particular electrophoretic variants, but also exhibit a common pattern of geographic variation, parallel selection provides the only satisfactory explanation.

As attempts to distinguish between the neutralist and selectionist hypotheses, studies of spatial patterns of variation have often proved to be ambiguous. In the absence of independent and accurate estimates of mutation rate, effective population size, gene flow or (in the case

of interspecific comparisons) time since divergence, both neutralist and selectionist arguments are sufficiently flexible to explain most observed patterns of variation. Moreover, since allelic variants can have identical electrophoretic mobility, electrophoretically detectable variation may represent only a fraction of the total variation⁷⁻¹⁰. A single electrophoretic mobility class ('electromorph'¹¹) may include many alleles with common mobility. Indeed, heterogeneity within single mobility classes has been demonstrated in several enzyme systems^{10,12-14}. Recognising this possibility, recent models which consider the behaviour of slightly deleterious alleles suggest that similarity of electromorph frequencies among apparently isolated populations or species may reflect independent approximations of the same mutation-selection equilibria rather than the effects of balancing selection^{11,15,16}. But the observation that electromorph frequencies vary in parallel in two sympatric species can only be explained by the latter hypothesis. Previous studies of enzyme variation in mussels¹⁷ and fish¹⁸ have documented possible examples of parallel response to environmental factors.

Gryllus veletis and *Gryllus pennsylvanicus* are sibling species of field crickets (Orthoptera, Gryllidae) found together in southern Canada, throughout the north-eastern United States, and south in the Appalachian Mountains to

Table 1 Mean PGI electromorph frequencies in *G. veletis* and *G. pennsylvanicus*

Electromorph	<i>G. veletis</i>	<i>G. pennsylvanicus</i>
PGI ¹⁶⁰	0.001	—
PGI ¹³⁵	0.040	0.105
PGI ¹²⁵	0.046	0.019
PGI ¹⁰⁰	0.804	0.797
PGI ⁶⁵	0.097	0.079
PGI ²⁵	0.010	—
<i>n</i>	40	52

Each value represents the unweighted mean of electromorph frequencies in 11 populations from the eastern United States. $\bar{n}$ is the average sample size (number of individuals). Localities are described in Fig. 1. The most common electromorph is designated 100 and other electromorphs are given numbers corresponding to their relative mobilities. Crickets were frozen soon after collection and stored at -80°C . They were thawed immediately before electrophoresis, the head and wings were removed and soluble proteins were extracted by homogenising legs, thorax and abdomen in 0.4–0.6 ml of 0.1 M Tris, 0.001 M EDTA, 5×10^{-5} M NADP, pH 7.0. Electrophoresis of phosphoglucose isomerase was on 12% starch gels using the discontinuous LiOH buffer of Selander *et al.*²⁶.

North Carolina¹⁹⁻²². They are exceedingly similar morphologically, have identical calling songs and are found in identical habitats^{19,21}. They can be distinguished only on the basis of differences in their life cycles²⁰⁻²⁵. *G. veletis* undergoes diapause as a late instar nymph and matures in May and June. *G. pennsylvanicus* undergoes diapause in the egg stage and adults appear in late summer.

Using starch gel electrophoresis combined with histochemical staining, I examined patterns of variation at 17

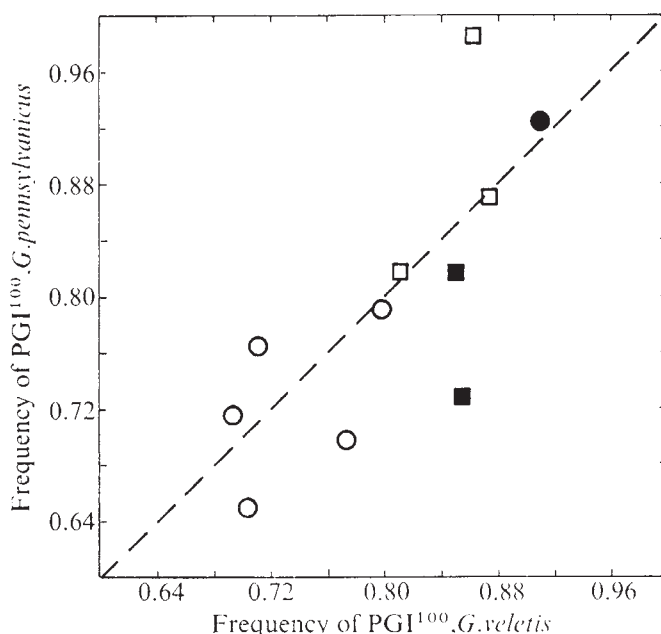


Fig. 1 Frequencies of PGI¹⁰⁰ in sympatric populations of *G. veletis* and *G. pennsylvanicus*. Each point represents a single collecting locality. Some localities were sampled in 1974, others in 1975. In no case, however, have data from more than 1 yr been combined. Along the dashed line electromorph frequencies are equal in the two species. $r_s = 0.82$, $P < 0.01$. r_s is the Spearman rank correlation coefficient. Localities: ○, Tompkins Co., New York; ●, Litchfield Co., Connecticut; □, Augusta Co. and Rockbridge Co., Virginia; ■, Alleghany Co., North Carolina.

loci in *G. veletis* and *G. pennsylvanicus* for evidence of parallel variation in electromorph frequency. Of these 17 loci, nine are essentially monomorphic in both species. Of the polymorphic loci, phosphoglucose isomerase (PGI) is unique in having nearly identical mean electromorph frequencies in the two species (Table 1). Analysis of PGI phenotypes in parents and offspring from single-pair matings confirm that the observed variation is genetic. Zygotic frequencies among the progeny are always consistent with segregation patterns predicted from the presumed parental genotypes (Table 2).

To test for the possibility of parallel variation at the PGI locus, I plotted the frequency of the most common electromorph, PGI¹⁰⁰, in eleven populations of *G. pennsylvanicus* against the frequency of the same electromorph in sympatric populations of *G. veletis* (Fig. 1). Samples were taken in the late spring (*G. veletis*) or late summer (*G. pennsylvanicus*) of 1974 and 1975. For each data point late instar nymphs or adults of both species were collected from identical localities. There is an obvious correlation in electromorph frequency ($r_s = 0.82$, $P < 0.01$, Spearman rank correlation coefficient). Moreover, the data points fall

Table 2 Genetic crosses of phosphoglucose isomerase variants in *G. veletis* and *G. pennsylvanicus*

Cross	Parental phenotypes	Species	100/160	Observed (expected*) No. of each phenotype among progeny			P†
				100/125	100	65/100	
1	100 × 100	p	—	—	7(7)	—	—
2	100 × 100	v	—	—	18(18)	—	—
3	100/160 × 100	v	23(17)	—	11(17)	—	0.1 > P > 0.05
4	100/125 × 100	v	—	8(8)	8(8)	—	> 0.9
5	100/125 × 100	v	—	16(15)	14(15)	—	0.9 > P > 0.5
6	65/100 × 100	p	—	—	17(17)	17(17)	> 0.9
7	100 × 65/100	v	—	—	11(15)	19(15)	0.5 > P > 0.1
8	100 × 25/100	v	—	—	10(9)	—	0.9 > P > 0.5
9	65 × 100	p	—	—	—	15(15)	—
10	65 × 100	p	—	—	—	14(14)	—

*Expected assuming normal Mendelian segregation with no dominance.

† χ^2 probability. χ^2 calculated by using correction for small sample size.

close to the line of equal frequency; that is, the frequency of PGI¹⁰⁰ tends to be the same in sympatric populations of the two species in spite of significant differences in electromorph frequency between allopatric populations of the same species. A plot of PGI⁸⁵ frequencies in the same sympatric populations of *G. veletis* and *G. pennsylvanicus* also yields a significant correlation ($r_s=0.66$, $P<0.05$).

All the collecting localities (including those within the same county) are thought to represent distinct populations. Both cricket species are predominantly short-winged (more than 99% in the populations examined), and dispersal by flight is rare. Evidence from mark-recapture studies indicates that adults do not disperse very far on foot, even within areas of favourable habitat. Moreover, among populations of *G. veletis* and *G. pennsylvanicus* from Tompkins County, New York, three of five polymorphic loci in each species exhibit significant heterogeneity in electromorph frequency (my unpublished results).

Hybridisation and introgression between the two cricket species cannot be invoked to explain the data. In spite of repeated attempts, hybrid progeny have never been produced in laboratory matings^{20-22,27}. Furthermore, I have found that natural populations of *G. veletis* and *G. pennsylvanicus* are fixed for different electromorphs at the phosphoglucosyltransferase-1 locus. No heterozygotes have been detected among 266 *G. veletis* and 206 *G. pennsylvanicus* examined.

The environmental factors responsible for the observed pattern of variation have not been identified. The correlation in electromorph frequencies, however, is in large part due to parallel changes in mean electromorph frequency between crickets from different geographic areas (Fig. 1). *G. veletis* and *G. pennsylvanicus* from Tompkins County, New York, have significantly lower frequencies of PGI¹⁰⁰ than populations from the mountains of Virginia. Crickets from Litchfield County, Connecticut, have distinctly higher frequencies of that electromorph. Superimposed on the regional pattern is considerable local variation. Although possibly due to selection, local variation may also result from random differentiation in relatively small populations with low interpopulation dispersal rates.

Two important questions remain unanswered. First, do the PGI¹⁰⁰ and PGI⁸⁵ electromorphs represent single allelic variants or are they heterogeneous assemblages of variants with common electrophoretic mobility? Second, does the PGI locus itself respond to environmental selection or is the observed pattern of variation the result of selection at a closely linked locus? If PGI¹⁰⁰ and PGI⁸⁵ represent classes of alleles with common mobility, then parallel variation may imply greater functional similarity of allozymes within a single mobility class than between classes. On the other hand, if PGI¹⁰⁰ and PGI⁸⁵ each represent a single allele, then linkage alone can explain the parallel variation if recombination frequencies between the PGI locus and the locus under selection are very small (because disequilibrium must have persisted in both species since their divergence). Answers to these questions must await detailed structural and functional characterisation of individual electromorphs.

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Possible role for acetylcholine in aggregation centre spacing in *Polysphondylium violaceum*

WHILE ridding my laboratory of an invasion of flies, I tested the toxicity of an organophosphate insecticide, 2,2-dichlorovinyl dimethylphosphate (DDVP), against the growth and development of *Polysphondylium violaceum* strain PvD 7206. Plate cultures of the slime mould exposed to the vapours of a pest strip containing the insecticide seemed to grow and develop normally in most respects, but showed a startling increase in the number of aggregation centres and sorocarps formed. I report here the results of preliminary investigations on the basis for this phenomenon.

A major biochemical effect of the organophosphates is inhibition of cholinesterase and/or other serine-esterases by phosphorylation of serine at the active site. DDVP seems to be a direct inhibitor, acting without prior metabolic conversion by the organism². The effect of exposure to the pesticide strip could be most nearly duplicated by exposing postvegetative cells deposited on membrane filters to filter pads saturated with a solution of 3×10^{-1} M DDVP (Fig. 1a, b). Concentrations below 1×10^{-4} M were not effective; concentrations above 5×10^{-3} M inhibited aggregation. It should be noted that the exposure conditions are not precisely equivalent. Cells exposed to the pesticide strip are under continuous assault from the vapours, whereas DDVP on filters is at maximum concentration only at the beginning of the exposure period. The compound is volatile and may evaporate from the filter surface. It is also subject to attack by phosphatases³ and may be actively degraded by the slime mould.

The choice of PvD 7206 (ref. 1) for the initial assay of the DDVP effect proved to be fortunate. In dense plate cultures this strain aggregates with some reluctance; confluent cultures form extensive tortuous streams, but may produce only a few decisive centres and large branched sorocarps. Exposure to DDVP vapours increases the number of centres produced in these conditions nearly 100-fold. Other stocks of *P. violaceum*, for example, PvD 7209 (ref. 1), normally aggregate avidly, producing hundreds of sorocarps, and the effect of DDVP is less spectacular. In populations of postvegetative amoebae allowed to develop on filters, there is little difference in the response of the two strains to DDVP; the number of centres is increased 2- to 4-fold in either case. The low frequency of centre emergence seen in confluent cultures of PvD 7206 may indicate that this strain is unusually sensitive to the centre-

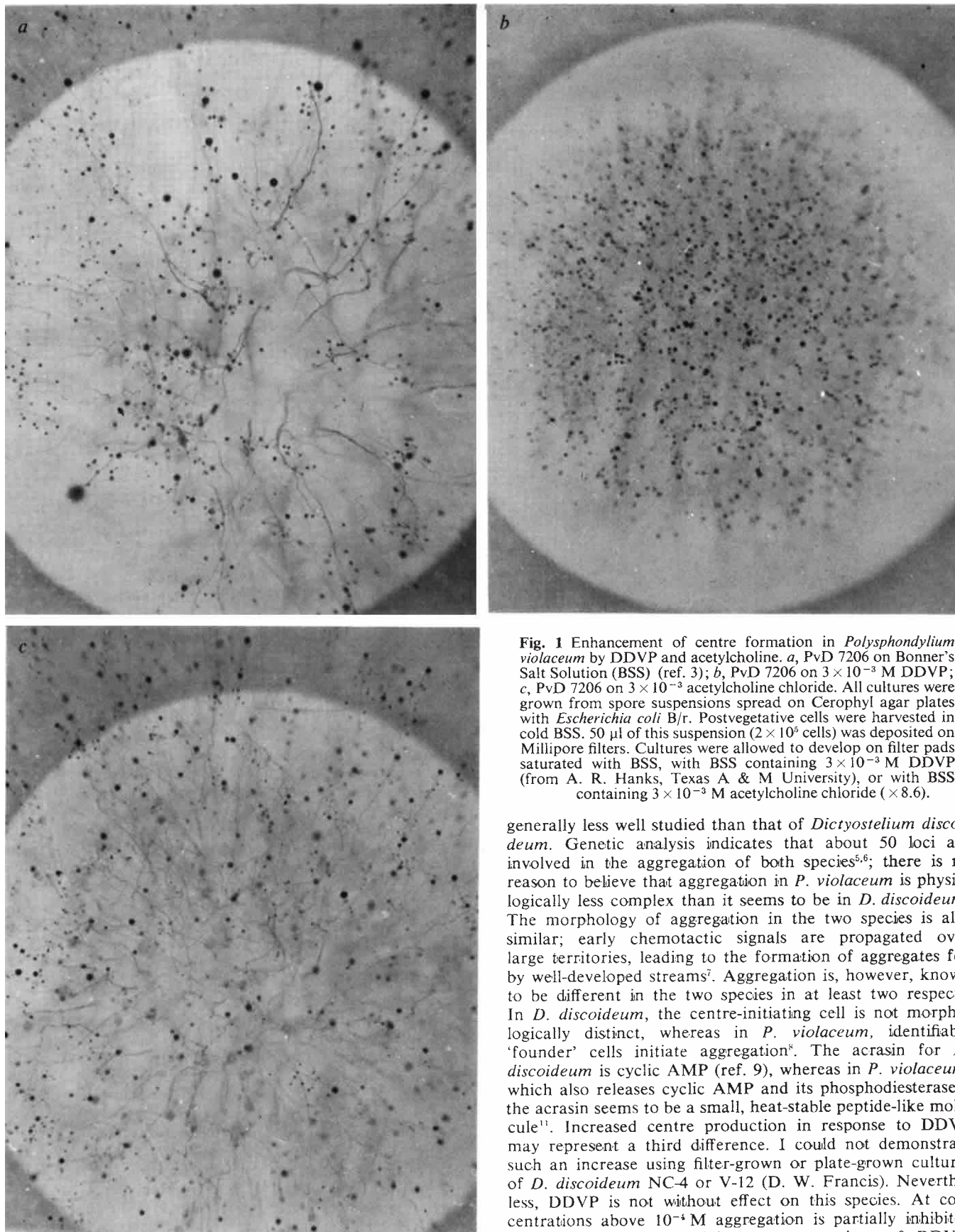


Fig. 1 Enhancement of centre formation in *Polysphondylium violaceum* by DDVP and acetylcholine. *a*, PvD 7206 on Bonner's Salt Solution (BSS) (ref. 3); *b*, PvD 7206 on 3×10^{-3} M DDVP; *c*, PvD 7206 on 3×10^{-3} acetylcholine chloride. All cultures were grown from spore suspensions spread on Cerophyl agar plates with *Escherichia coli* B/r. Postvegetative cells were harvested in cold BSS. 50 μ l of this suspension (2×10^5 cells) was deposited on Millipore filters. Cultures were allowed to develop on filter pads saturated with BSS, with BSS containing 3×10^{-3} M DDVP (from A. R. Hanks, Texas A & M University), or with BSS containing 3×10^{-3} M acetylcholine chloride ($\times 8.6$).

inhibiting mechanism involved in spacing in this species. Inhibition of centre formation by a diffusible substance emanating from previously established centres has been demonstrated in *P. violaceum*⁴, but the inhibitor has not been identified.

The physiology of aggregation in *P. violaceum* is

generally less well studied than that of *Dictyostelium discoideum*. Genetic analysis indicates that about 50 loci are involved in the aggregation of both species^{5,6}; there is no reason to believe that aggregation in *P. violaceum* is physiologically less complex than it seems to be in *D. discoideum*. The morphology of aggregation in the two species is also similar; early chemotactic signals are propagated over large territories, leading to the formation of aggregates fed by well-developed streams⁷. Aggregation is, however, known to be different in the two species in at least two respects. In *D. discoideum*, the centre-initiating cell is not morphologically distinct, whereas in *P. violaceum*, identifiable 'founder' cells initiate aggregation⁸. The acrasin for *D. discoideum* is cyclic AMP (ref. 9), whereas in *P. violaceum*, which also releases cyclic AMP and its phosphodiesterase¹⁰, the acrasin seems to be a small, heat-stable peptide-like molecule¹¹. Increased centre production in response to DDVP may represent a third difference. I could not demonstrate such an increase using filter-grown or plate-grown cultures of *D. discoideum* NC-4 or V-12 (D. W. Francis). Nevertheless, DDVP is not without effect on this species. At concentrations above 10^{-4} M aggregation is partially inhibited or delayed. At non-inhibitory concentrations of DDVP, centre and slug formation seem normal, but at culmination the slug tip regresses, the sorogens become club-shaped and may arrest without further differentiation or produce sorocarps with relatively short stalks. Slug migration is also suppressed. Hanks (personal communication) has noted that another organophosphate, malathion, both accelerates

aggregation and increases the number of centres formed in submerged populations of *D. discoideum* Ax-2. Whether this represents a response similar to that of *P. violaceum* to DDVP has not been established. It may be a unique consequence of the use of malathion, the difference in assay conditions, or the use of Ax-2; other idiosyncratic responses of the axenic strains have been noted¹². Some organophosphates may, by acting as phosphate donors competitive with ATP, intervene in the membrane phosphorylation that precedes and has been reported to accelerate aggregation in *D. discoideum* (ref. 13).

The increased centre production by *P. violaceum* in the presence of DDVP suggests that cholinesterase or another serine-esterase is instrumental in determining the size of the aggregation territory in this species. The possible existence of a cholinergic communication system in this cellular slime mould is appealing in view of the role of acetylcholine in synaptic transmission, and the suggestion of McMahon¹⁴ that neurotransmitters may function as developmental regulators. Preliminary evidence that the DDVP effect is at least in part a consequence of interference with a cholinergic mechanism is summarised in Table 1. Organophosphate inhibition of cholinesterase might be expected to facilitate an increase in the concentration of acetylcholine. Postvegetative cells of *P. violaceum* 7206 exposed to exogenous acetylcholine (3×10^{-3} M) showed an increase in centre production similar to that seen with DDVP. Culmination of these centres may, however, be incomplete, as the number of sorocarps produced seems to be intermediate (Fig. 1c); acetylcholine-treated populations produce more sorocarps than control populations, but fewer sorocarps than DDVP-treated populations. Atropine, an acetylcholine antagonist, reduces or inhibits centre formation. Eserine, a known inhibitor of cholinesterase, also stimulates centre formation, although its effect is less striking than that of DDVP. The difference may reside in their mode of action; eserine binds reversibly to cholinesterase, while the dimethyl phosphate of DDVP is covalently attached to the enzyme². DDVP may also have effects other than those related to cholinesterase inhibition. These observations suggest that acetylcholine reduces territory size in *P. violaceum* and that cholinesterase increases it. Further speculation about the mechanism of this activity and its relationship to cyclic nucleotide metabolism and the ionic fluxes of aggregation should be reserved until the elements of a cholinergic system are isolated and un-

equivocally identified. *P. violaceum* synthesises several DDVP-sensitive enzymes with esterase activity (Clark, McCoy, and Whitmore, unpublished observations); an effort to associate one or more of these enzymes with the centre-increasing effect of DDVP, and to identify a cholinesterase among them, has been initiated.

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Frequency of mating-type switching in homothallic fission yeast

THE genes determining mating type in the fission yeast *Schizosaccharomyces pombe* are of particular interest in the general study of the genetic control of differentiation. The genetic segments concerned, *mat1^M* and *mat2^P*, specifying mating type (–) and (+), respectively¹, are present, closely linked, in homothallic strains², but on any one chromatid (that is in the *cis* position) they are only expressed in a mutually exclusive manner³. The segments of the mating-type locus, *mat1^M* and *mat2^P*, govern the transition from vegetative growth to sexual reproduction. They act in a complementary fashion—one functional *M* segment and one functional *P* segment are always required to initiate both conjugation (usually among haploid cells) and meiosis (leading to sporulation in either zygotes or diploid cells), the prominent events in differentiation in *S. pombe*. A mating-type mutant defective in meiosis but not in conjugation (*mat1^M mat2^{Pm}*) when crossed with wild type, produces sporulating and non-sporulating zygotes (Fig. 1). These two classes indicate that in individual cells of either parent strain only one or the other mating-type segment is active³. Here I describe a single-cell evaluation of this cross, and show (also by single-cell techniques) that the frequency of mating-type switching in haploid and diploid strains is approximately random. A favoured mechanism for this type of switching is the inversion of a promoter element between the structural genes, the direction of the promoter determining which mating-type segment is switched on.

Cells of *S. pombe* are usually haploid during vegetative growth. It is only when the culture medium becomes exhausted, particularly of nitrogen, that diploid zygotes are formed by pairwise cell fusion. If left undisturbed, these zygotes undergo meiosis and sporulation, thereby immediately regenerating the haploid state. Yet it is possible to induce diploid division in a small fraction of wild-type zygotes or very efficiently in certain mutants⁴. When Leupold introduced *S. pombe* into basic genetics (see ref. 2), his strain already consisted of a mixture of heterothallic clones, in which either mating type ((+) or (–)) is heritably stable or nearly so, and of homothallic ones, in which conjugation is possible even among sister cells. Heritable changes of mating type occur in various directions¹⁻³, usually of the order of 10^{-4} per cell division;

Table 1 Effect of various additives on centre production of *P. violaceum*

Additive	Relative no. of centres formed
BSS	1.0
DDVP	
3×10^{-3} M	4.0
1×10^{-8} M	2.5
Acetylcholine	
3×10^{-3} M	3.5
1×10^{-3} M	1.1
Atropine	
3×10^{-3} M	0
1×10^{-3} M	0.6
Eserine	
1×10^{-3} M	2.6
1×10^{-4} M	1.6

Filter cultures were prepared as indicated in Fig. 1, except that some experiments were done using drop sizes at 10 or 20 μ l for easier centre counting. Number of centres was determined by counting the average number of centres formed on three filters. Data from several independent experiments were combined for comparison by setting all figures relative to an arbitrary control (BSS) value of 1.0. The number of centres produced by control cultures averaged 17 for 10- μ l drops and 90 for 50- μ l drops. Variation for any set of testing conditions was 20% or less.

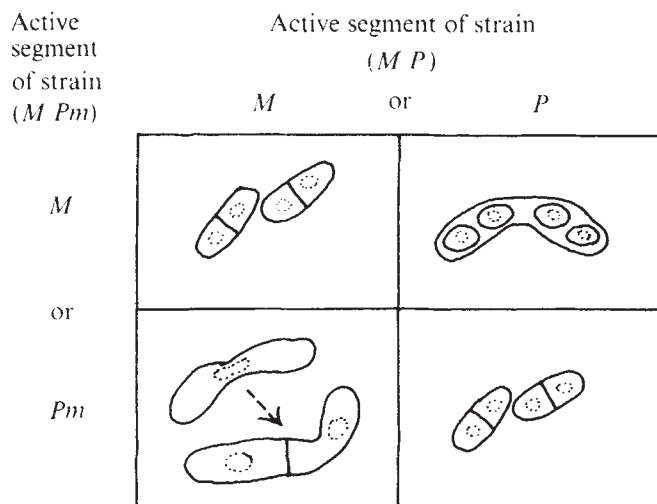


Fig. 1 Zygote classes of cross $(M Pm) \times (M P)$. On the assumption that cells of either parent strain can only activate one *mat* segment or the other, the possible four pair-wise combinations are indicated by this scheme. Two cells expressing the same mating type do not interact and, at best, divide again mitotically. Two cells expressing opposite mating types can either sporulate, if the (+) cell is wild type, or not, if the (+) cell is mutant for the *Pm* allele. Since the genetic block of this mutation is effective before commitment to meiosis⁴ and before premeiotic DNA replication¹⁰, most zygotes of the latter class can be induced to resume diploid division by a transfer to growth medium.

this is more frequent than ordinary mutations, though well below the rapid switching reported here.

In the cross of $(M Pm) \times (M P)$, illustrated in Fig. 1, both parent strains are homothallic and can produce zygotes by selfing, in addition to the really informative zygotes of hybrid descent. Colonies originating from the various classes of zygote can be distinguished by the expression of marker genes (two *ade6* alleles in this cross) (Table 1). Evidently selfing (I, II) was still preferred over crossing (III, IV); but the hybrid colonies (III compared with IV) are compatible with a 1:1 distribution with regard to the two classes of zygote indicated in Fig. 1. A few non-sporulating zygotes in fact give rise to segregating haploid colonies rather than to diploid ones, leading to incidental misclassification. Some additional uncertainty is introduced by the unclassified number of zygotes (V) that failed to form colonies. The colonies produced by class III zygotes contain sporulating and non-sporulating diploid cells. This aspect is discussed below.

The next point of interest is the frequency with which cells can alternate their choice as to which mating-type segment is to be

Table 1 Colonies from zygotes of cross $(M Pm) ade6-M216 \times (M P) ade6-M210$

Colour	Colonies	Interpretation
I Light red	109	diploid, homozygous for <i>M216</i> ; selfing of $(M Pm)$
II Dark red	68	haploid, <i>M210</i> ; selfing of $(M P)$
III White	28	diploid, heterozygous <i>M-216/M210</i> ; cross, diploidised zygotes
IV Streak-sectored, light/dark red	37	haploid, <i>M216</i> and <i>M210</i> ; cross, sporulated zygotes
V Zygotes that failed to grow	76	?

Cells of the strains $(M Pm) ade6-M216$ and $(M P) ade6-M210$ were crossed by the shift method¹⁰ (transfer to liquid medium devoid of a nitrogen source). After agglutination had just started, the culture was briefly sonicated to reduce the chance of selfing in either parent strain. Two hours later, samples were streaked on agar slabs, and individual zygotes were isolated by micromanipulation. The slabs were then transferred to YEA plates (yeast extract agar, unsupplemented with adenine) and incubated for colony production. On this medium, both *ade6* alleles used in this cross can be distinguished by different intensities of a pink pigment. No pigment is formed in heterozygous diploid colonies, owing to allelic complementation¹¹.

expressed. Unfortunately, clear-cut tests for both (+) and (−) mating-type activities of single cells, such as have been worked out for budding yeast⁵, are not yet feasible in *S. pombe*.

In the experiment considered here, a haploid, homothallic strain has been used that is wild type at its mating-type locus (*M P*) but mutant at two other genes, *fus1* and *cdc2*. Conjugation is initiated as usual, but cell fusion is prevented by the *fus1* mutation⁴. The *cdc2* marker is a temperature-sensitive cell cycle mutation blocked in nuclear division⁶.

The rationale was as follows. Haploid cells affected by *fus1* could at best form 'prezygotes', pairs of cells recognisable by their attached conjugation tubes. The asci observed after high-temperature exposure could only have arisen by endodiploidisation (provoked by *cdc2*). The sister chromatids emerging from the previous replication are distributed to sister cells if division is possible, or remain within the same cell after endodiploidisation. Since different mating-type segments must be activated on sister chromatids in order to produce a pair of prezygotic cells after one division, or a single ascus without division, these events are suitable indicators of mating-type switching.

Results are given in Table 2. If the frequency of switching were high enough for one mating-type segment or the other to be

Table 2 Oppositely activated mating-type segments on sister chromatids of strain $(M P) cdc2 fus1$

	Hours at 36 °C		
	0–2.5	3.5–5	20
Total	451	184	56
1n	322	131	40
2pz	68	(5)	—
1a	(1)	34	—
Fraction	2pz/1n = 0.21	1a/1n = 0.26	

1n, uninucleated cells at plating, as calculated from the fraction of single cells in the last column;

2pz, one pair of prezygotic cells produced by the first division;

1a, single ascus, produced without division.

Haploid cells of the strain $(M P) fus1 cdc2$ were cultured in liquid sporulation medium supplemented by a nitrogen source⁵. Cells were then plated on agar medium of the same composition but lacking the nitrogen source. Only one or two residual divisions are usually observed on this medium before induction of conjugation and/or meiosis. The plates were incubated at 36 °C for different lengths of time and shifted back to 30 °C. About 20 h after plating, all plates were inspected microscopically, and the different combinations of prezygotic cells, asci, or neutral cells that had been formed after one or two residual divisions were counted. The most informative configurations (2pz and 1a) are represented here. One plate (last column) was incubated at 36 °C throughout to calculate the fraction of plated cells that had been arrested without division at the non-permissive temperature (corresponding to the uninuclear fraction at the time of plating).

activated at random on any one chromatid, just half the uninucleate cells plated to sporulation medium should develop to a single ascus or a pair of prezygotic cells. The observed frequencies reach about half this expectation, and may actually be closer to randomness if the efficiency of conjugation induction or of endodiploidisation is less than 100%.

The third experiment was designed to estimate frequencies of switching in diploid cells heterozygous for $(M Pm)/(M P)$, which had been derived from the first cross (Table 1, class III). This test was performed as a pedigree analysis of cells on a growth-limiting and sporulation-inducing medium. In principle, each newly separated cell had three choices as depicted in Fig. 2. The rationale was that the different combinations of activated *mat* segments, which are indicated for separate haploid cells by Fig. 1, are now carried by individual diploid cells. A cell could develop into an ascus, provided that mating-type segments were properly activated in the wild-type combination, or develop the 'premeiotic' phenotype peculiar to the *Pm* mutation; or else it could divide again mitotically. The first two possibilities represent the alternative choices of complementary mating-type activation. The pattern by which these configurations are distributed through the

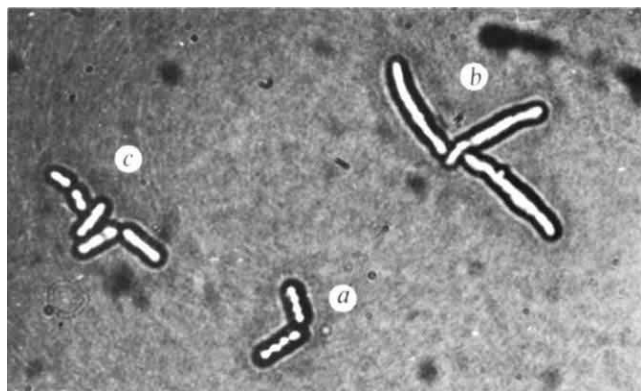


Fig. 2 Three choices of heterozygous (*M Pm*)/(*M P*) cells. Diploid cells of a heterozygous (*M Pm*)/(*M P*) strain were plated to sporulation medium, and observed after being incubated overnight. The progenies of three individual cells are depicted in this illustrative, though highly selected view, in which descendants of any one cell happened to be identical. *a*, Two asci have developed after one division; *b*, three 'premeiotic' cells have resulted from two divisions; *c*, five still vegetative-looking cells have been formed by four divisions. These possibilities were used to infer the states of activation for the different *mat* alleles during pedigree analysis. The 'premeiotic' phenotype, attributable to the *Pm* mutation, is equivalent to the aberrant elongation generally observed when DNA replication is selectively inhibited.

pedigrees should reveal for how many divisions a given combination is propagated, or how frequently the cells can switch.

Data of this analysis are given in Table 3. In *a*, single cells are evaluated with respect to the three choices listed above. The observed distribution is somewhat skewed in favour of dividing cells and premeiotic cells. In *b*, cell pairs (sister cells) are evaluated, and compared with the expectation based on a random choice after each division and biased by the skewed distribution seen in *a*. Evidently, even pairs (cc, pp, aa) are favoured over odd pairs (pc, ac, pa). Hence, a low degree of heritability has to be assumed for the different combinations of activated *mat* segments. On the other hand, the 'pa' pairs (an ascus formed next to a premeiotic cell) deviate relatively little from the expected value.

A random-choice distribution corresponds to an average frequency of 0.5 switches per chromatid and division. If the actual frequency were half that value, the expected value for a double event (changes on both chromatids), such as the formation of a 'pa' pair, should have dropped to a quarter: from 75 to 19. This value is considerably lower than the observed 58 pairs of this kind.

Randomness as to which *mat* segment is activated on any one chromatid is therefore fairly well approximated in both haploid and diploid cells of homothallic *S. pombe* strains. The *cis*-acting functional exclusion of either mating-type segment by the other

can best be explained by intra-strand recombinational models that allow for rearrangements of structural genes relative to their controlling signals. Since, in *S. pombe* at least, the respective silent segment of the mating-type locus is still located at its original position on the chromosomal map, a flip-flop model by means of an invertible promoter element between the structural genes of the *mat1^M* and *mat2^P* segments is favoured for this yeast⁷: the relative direction of the promoter element then determines the mating-type segment to be activated in conditions for conjugation. Such a scheme has the advantage that a variety of genetic instabilities, all localised at the mating-type locus¹⁻³, can be attributed to a common mechanism of site-specific recombination. There are, of course, other explanations for a random-choice mechanism, but mutants have been described⁸ that seem to reduce the rates of switching, so that sizeable clones of one mating type or the other are produced, which can best be explained by reversible alterations at the level of the gene.

The independent control of mating-type switching on both chromosomes of diploid *Schizosaccharomyces* cells is at variance with homothallism in *Saccharomyces*, where switching ceases after conjugation, and the resulting diploid cells stably transmit a once assumed *a/α* configuration⁹ (*a* and *α* are the mating types of that yeast). This discrepancy seems to be related to the different life cycles of both yeasts. Most budding yeasts have evolved to a stable diploid growth phase by means of conjugating under growth conditions and zygotic budding, whereas fission yeasts do not usually grow as diploids, since conjugation is confined to starvation and is followed by zygotic reduction and sporulation. Whereas the latter condition does not require a special stabilisation for the diploid state, continued mating-type switching is incompatible with the life cycle of a budding yeast, since it would inevitably lead to excessive polyploidisation.

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Table 3 Pedigrees of diploid (*M Pm*)/(*M P*) cells

<i>a</i>	<i>c</i>	<i>p</i>	<i>a</i>	Total	<i>za</i>
Observed	1322	488	328	2138	(32)
Fraction	0.62	0.23	0.15		
<i>b</i> , pairs	<i>cc</i>	<i>pp</i>	<i>aa</i>	<i>pc</i>	<i>ac</i> <i>pa</i>
Observed	494	136	47	158	176 58
Expected	408	56	25	302	203 75

c, Cell divided again.

p, Premeiotic cell.

a, 'Azygotic' ascus (sporulated cell with haploid spores).

za, 'Zygotic' ascus (sporulated zygote with diploid spores).

Diploid (*M Pm*)/(*M P*) cells were cultured and streaked to agar slabs as indicated below Table 2. Contrary to the previous experiment, however, more than two residual divisions were possible this time, owing to some impurities in a new batch of agar. Pedigrees were started by isolating cells that had just divided. For several generations, sister cells were separated by micromanipulation. The slabs were then incubated overnight and evaluated microscopically. Since conspicuous clustering of any sort was not noticed in these pedigrees, only pairs of sister cells are considered here, and the data are pooled for the first four rounds of cell division. Occasionally, 'zygotic' asci had been formed by inseparable sister cells; but these have not been considered further, since their interpretation is ambiguous.

Rapid, chloramphenicol-resistant, activation of membrane electron transport on germination of *Bacillus* spores

KEILIN¹ observed that the life cycle of diverse organisms includes a stage when life seems to be arrested, which he termed the cryptobiotic state. As an example of this, the mature bacterial spore can remain dormant with no detectable metabolism for long periods and then on exposure to germinants, rapidly germinate and outgrow in a synchronous manner, to yield a vegetative cell. We have been particularly interested in the composition and activities of the membrane-bound electron transport chain in sporulating *Bacillus megaterium* KM (ref. 2) and in the unique reversed orientation which characterises the membranes surrounding the developing spore³. This report describes the rapid changes observed in the membrane of *Bacillus megaterium* KM spores during germination and outgrowth. A rapid chloramphenicol-resistant, activation of membrane NADH oxidase activity occurred within 5–10 min of germination. Membrane-bound DL-α-glycerol-P and L-malate oxidase activities appeared sequentially at later times and their appearance was prevented by

Table 1 Development of inner membrane oxidase activities during spore germination and outgrowth

Time of germination (min)	Oxidase activity (nmol of O ₂ per min per mg protein)					
	NADH		DL- α -Glycerol-P		L-malate	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
Dormant spore (0)	48	—	< 2	—	< 2	—
Heat activated spore (0)	—	85	—	< 2	—	< 2
10	—	639	—	2.6	—	< 2
20	554	676	9.8	4.3	< 2	< 2
50	728	718	39	30	2.2	2.8
100	806	1418	44	113	13	18

The sporulation medium (CCY medium) used to produce spores was a modification of that used previously^{14,15}. This contained MgCl₂·6H₂O (0.5 mM); MnCl₂·4H₂O (0.01 mM); FeCl₃·6H₂O (0.05 mM); ZnCl₂ (0.05 mM); CaCl₂·6H₂O (0.2 mM); KH₂PO₄ (30 mM); K₂HPO₄ (10 mM); glutamine (20 mg l⁻¹); acid casein hydrolysate (1 g l⁻¹); enzymatic casein hydrolysate (1 g l⁻¹); enzymatic yeast extract (0.4 g l⁻¹) and glycerol (0.6 g l⁻¹). Mature spores liberated on completion of sporulation in this medium were collected by centrifugation and repeatedly washed in deionised water (4 °C). Spore suspensions (50 mg dry weight ml⁻¹) were heat-activated in deionised H₂O at 70 °C for 30 min, allowed to cool to room temperature then resuspended in modified CCY medium plus germinants (1 mM L-alanine + 5 mM inosine) to a density of about 0.5 mg dry weight ml⁻¹. The suspensions were incubated at 30 °C on an orbital shaker (220 r.p.m.) for various times before being chilled by addition of ice cubes followed by rapid centrifugation at 4 °C. The organisms were resuspended in 0.05 M Tris-HCl, pH 7.5 (4 °C), mixed with cooled glass beads and broken in the Braun cell disruptor for 1 min with liquid CO₂ cooling. Spore integuments and occasionally a few unbroken organisms were removed by centrifuging (25,000g for 3 min at 4 °C) and the resulting supernatant was centrifuged at 105,000g for 1 h at 4 °C to deposit the membrane fraction. The membrane fraction was washed once by re-suspension in 0.05 M Tris-HCl (4 °C) containing 5 mM MgCl₂. Oxidase activities at 30 °C were determined as described previously². *a* and *b* refer to separate experiments.

chloramphenicol. Evidence is presented to suggest that in the dormant spore the electron transport sequence is blocked in the region between NADH dehydrogenase and the cytochromes.

Table 1 shows that both dormant and heat-activated spore membranes had essentially the same low NADH oxidase activity. Within 5–10 min of germination there was a 5–10-fold stimulation of NADH oxidase activity (Tables 1 and 2) that probably increased further during the germination and outgrowth sequence. L-malate oxidase activity was first detectable after 50 min and had not reached full vegetative cell membrane levels of activity (approximately 50 nmol O₂ min⁻¹ mg⁻¹) after 100 min. DL- α -glycerol phosphate oxidase activity was detectable after 10 min and attained approximately vegetative cell membrane levels of activity (70 nmol O₂ min⁻¹ mg⁻¹) during the experimental period. DL- α -glycerol-P dehydrogenase activity showed a similar pattern (Table 2).

It was of interest to see whether these changes in oxidase activities were the result of protein synthesis or activation of pre-existing proteins. Steinberg *et al.*⁴ had reported a rapid, chloramphenicol insensitive increase in oxygen uptake in the first minutes

of *B. cereus* spore germination. In an experiment where the dormant spore membranes had an NADH oxidase activity of 67 nmol O₂ min⁻¹ mg⁻¹ protein, the membranes of organisms germinated for 10 min in the presence or absence of chloramphenicol (100 μ g ml⁻¹) had activities of 723 and 855 nmol O₂ min⁻¹ mg⁻¹ protein, respectively. This level of chloramphenicol completely inhibits protein synthesis during germination of *B. megaterium* spores⁵. When spores were germinated with chloramphenicol for longer periods (Table 2), DL- α -glycerol P and L-malate oxidase activities did not appear, indicating a requirement for protein synthesis for incorporation of these activities into the membrane. Once again, the initial rapid increase in NADH oxidase activity was unaffected by chloramphenicol but the activity subsequently decreased after 20 min (see Table 2), indicating new protein synthesis to be necessary to maintain this level of activity later in the germination sequence.

To determine whether the NADH oxidase activation occurred generally over the whole electron transport sequence, or whether a specific portion was stimulated, certain segments of the sequence were assayed using artificial electron donors and acceptors (Table 2). These experiments revealed that heat activation had no significant effect on the activity of any of the enzymes in Table 2: NADH-DCPIP reductase activity was higher than NADH oxidase activity and did not change significantly on germination; NADH-cytochrome *c* reductase activity showed a dramatic increase on germination, of the same order as the increase in NADH oxidase; and that ascorbate-TMPD oxidase activity was low and did not increase on germination. These results suggest that the electron transport sequence of the dormant spore membrane is blocked between the dehydrogenase and cytochrome *b* regions.

Room temperature reduced minus oxidised difference spectra did not reveal any rapid change in membrane cytochrome content during germination. NADH reduced aerobic minus oxidised, liquid nitrogen temperature spectra revealed a lower steady state level of reduction of the cytochromes in the dormant spore membranes than in membranes from spores germinated for 10 min, which together with the assays of the segments of the electron transport sequence, is consistent with a block located in the sequence before the cytochromes.

When spores were germinated in phosphate buffer plus germinants immediately after heat activation (Fig. 1), oxygen uptake measured in the oxygen electrode was first detectable after about 2 min. The rate of uptake then increased rapidly, so that by 7 min the suspension was respiring vigorously (25.8 nmol O₂ min⁻¹ mg⁻¹ dry weight of spores). This oxygen uptake did not occur in the absence of germinants and was unaffected by chloramphenicol. The results were the same for spores germinated in the more complex modified CCY medium (Fig. 1). Inclusion of KF (10 mM) in the germination medium slightly delayed the onset of rapid respiration but the final rate attained was little different from that of control suspensions, as described previously⁶. With KCN (10 mM) present, the maximum rate of respiration was approximately half that of the controls. The rapid rate of respiration attained within the first 7 min of germination corresponds to that

Table 2 Assays of segments of the electron transport chain during germination

Time of germination (min)	NADH-oxidase			NADH-2,6-dichlorophenol-indophenol reductase			NADH-cytochrome <i>c</i> reductase			Ascorbate-TMPD oxidase			DL- α -glycerol-P-oxidase			DL- α -glycerol-P-2,6-dichlorophenol-indophenol reductase		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
Dormant spore	99	44	—	1.68	1.79	—	69	25	—	28	43	—	< 2	< 2	—	5	9.7	—
Heat-activated spore	74	—	40	1.06	—	2.38	28	—	33	26	—	41	< 2	< 2	< 2	2	6.5	15
5	377	239	—	1.9	1.44	—	175	160	—	39	39	—	< 2	< 2	—	7	4.4	—
10	425	416	400	2.0	1.68	1.45	182	159	159	28	45	31	5.8	4.8	< 2	8	19.6	9
20	1048	415	202	2.3	1.28	0.79	190	135	120	53	26	26	39	16	< 2	80	45.1	5

The experimental details were essentially as in Table 1. Enzyme assays were carried out at 30 °C as described previously^{2,3}. Assays of ascorbate-TMPD oxidase contained 10 mM ascorbate and 0.1 mM TMPD. Correction was made for the low non-enzymatic oxidation of ascorbate-TMPD. *a* and *b* refer to separate experiments; in *c*, the germination was carried out in the presence of chloramphenicol (100 μ g ml⁻¹). Enzyme activities are expressed in the following units: NADH oxidase (nmol O₂ min⁻¹ mg⁻¹ protein); NADH-2,6-dichlorophenol-indophenol reductase (μ mol 2,6-dichlorophenol-indophenol reduced min⁻¹ mg⁻¹ protein); NADH-cytochrome *c* reductase (nmol cytochrome *c* reduced min⁻¹ mg⁻¹ protein); Ascorbate-TMPD oxidase (nmol O₂ min⁻¹ mg⁻¹ protein); DL- α -glycerol-P oxidase (nmol O₂ min⁻¹ mg⁻¹ protein); DL- α -glycerol-P-2,6 dichlorophenol-indophenol reductase (nmol 2,6 dichlorophenol-indophenol reduced min⁻¹ mg⁻¹ protein).

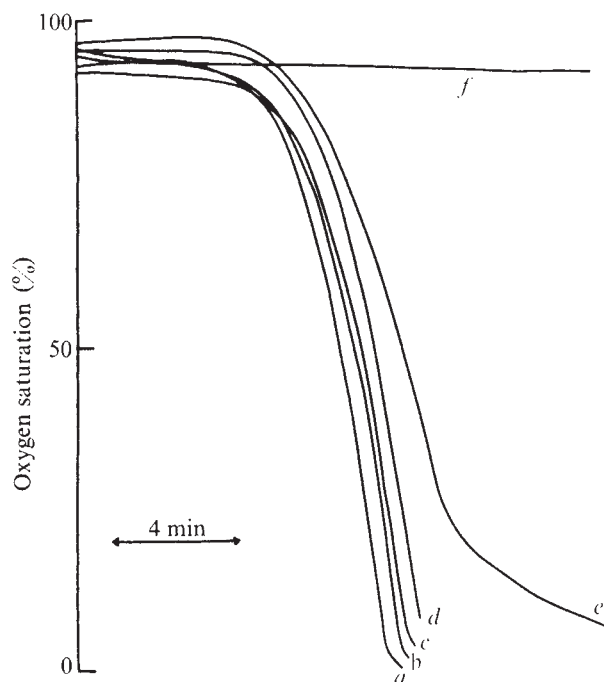


Fig 1 Aliquots of washed concentrated spores in deionised water ($10 \text{ mg dry weight ml}^{-1}$) were heat activated by heating in a water bath at 70°C for 30 min. Immediately after heat activation, 0.2 ml of this spore suspension was added to the reaction vessel of the oxygen electrode. Incubations were as follows: 50 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7.5) containing 1 mM L-alanine and 5 mM inosine (b); c, d and e repeats of (b) with addition of $100 \mu\text{g ml}^{-1}$ chloramphenicol, 10 mM potassium fluoride and 10 mM potassium cyanide, respectively; f, result of incubation in 50 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7.5) alone. The final incubation volume was 3 ml in all cases and the reaction vessel temperature was maintained at 30°C .

observed by Steinberg *et al.*⁴ and correlates with the observed increase in membrane NADH oxidase activity.

This rapid chloramphenicol-insensitive activation of the respiratory membrane of the spore on germination seems to be a general property of *Bacillus* spores, since similar results to those described here have been obtained for all species so far examined including *B. cereus* and *B. subtilis*. The activation also occurs in the absence of membrane lipid synthesis which is not detectable until 10 min after germination⁷. When spores were germinated in 40 mM Tris-HCl buffer containing either 1 mM L-alanine or 40 mM calcium-dipicolinic acid⁸, the same pattern of NADH oxidase activation was observed. Since, in these conditions the spores could germinate but not outgrow, it would seem that the activation is not inextricably linked to spore outgrowth.

The biochemical basis of the suggested block in the dormant spore electron transport chain now remains to be determined. Possible mechanisms to account for its rapid removal during germination include degradation or dissociation of an inhibitor and membrane conformational changes resulting from the expansion of spore membrane which is thought to occur early in germination. Although lipid synthesis is not detectable until 10 min after germination is initiated, turnover of existing spore membrane lipids starts almost immediately on initiation and is therefore occurring the period of NADH oxidase activation⁷.

Rapid increases in respiratory rates upon termination of the cryptobiotic stage have also been recorded for fungal spores⁹, and insects in diapause¹⁰. To what extent activation of pre-existing enzymes is involved in these instances is not known. Activation dose seem to have a role in sea urchin eggs after fertilisation, where the sudden increase in respiratory activity was found to be insensitive to chloramphenicol and actinomycin D (refs 11 and 12). In etiolated primary leaves of *Phaseolus vulgaris*, green chloroplasts formed during exposure to short light flashes did not exhibit Photosystem II activity. These chloroplasts, however, acquired the full Photosystem II activity within minutes after exposure to

continuous light, suggesting that some form of activation is occurring¹³. It is not known whether this activation is independent of protein synthesis.

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Solubilisation of atropine-binding material from brain

MUSCARINIC receptors have been characterised extensively in intact smooth muscle^{1–3} and in subcellular fractions from smooth muscle^{2,4–6} and brain^{7–10} by reversible binding of labelled atropine^{1,9,10}, propylbenzylcholine^{9,10}, quinuclidinylbenzilate (QNB) (refs 6, 8) and benzetimide^{4,5}, or by alkylation with benzylcholine² or propylbenzylcholine mustard^{3,7,9–11}. Several attempts to solubilise muscarinic receptors^{2,4,12} have failed due to the inhibition of receptor binding by the detergent used for solubilisation^{2,4}. We report here the solubilisation with 2M sodium chloride of an atropine-binding material from the ox cerebral cortex, which has properties similar to those found for the muscarinic receptor in subcellular preparations.

Figure 1a shows the binding of ($\pm$) ³H-atropine to a crude synaptosomal preparation isolated from the cerebral cortex; the measured dissociation constant (K_D) was $2.0 \pm 0.3 \text{ nM}$, the Hill coefficient n_H was 1.0 and the concentration of the binding sites was $2.0 \pm 0.5 \text{ nmol g}^{-1}$ protein. The high affinity binding of labelled atropine could be displaced by unlabelled atropine, scopolamine, lachesine and QNB. The estimates of the inhibition constants (K_i) of these antagonists, together with those of agonists (determined in this study and by other groups) are shown in Table 1. There was close agreement between the constants measured in our preparations and those reported by others although, in our hands, QNB showed two distinct affinities ($K_i = 2 \times 10^{-10} \text{ M}$ and $3 \times 10^{-8} \text{ M}$) of which only the higher affinity value has been reported before⁸, possibly because its binding had not been studied at sufficiently high concentrations.

Figure 2 illustrates the inhibition by acetylcholine of binding of labelled atropine to the membrane preparations. The inhibition curve indicates two binding sites for acetylcholine: a high affinity site with $\text{ID}_{50} = 10^{-5} \text{ M}$ and a lower affinity site with $\text{ID}_{50} = 10^{-3} \text{ M}$. These values correspond to values of K_i of $3 \times 10^{-6} \text{ M}$ and $3 \times 10^{-4} \text{ M}$, respectively. Three other agonists,

acetyl- β -methylcholine, oxotremorine and carbamylcholine gave similar biphasic curves for inhibition of atropine binding. Their affinity constants derived from the competition with ^3H -atropine were respectively 3×10^{-6} M and 10^{-4} M, 3×10^{-7} M and 2×10^{-5} M, and 2×10^{-6} M and 2×10^{-4} M. We found that the high-affinity binding component represented 35–45% of the total number of atropine-binding sites, a figure similar to the

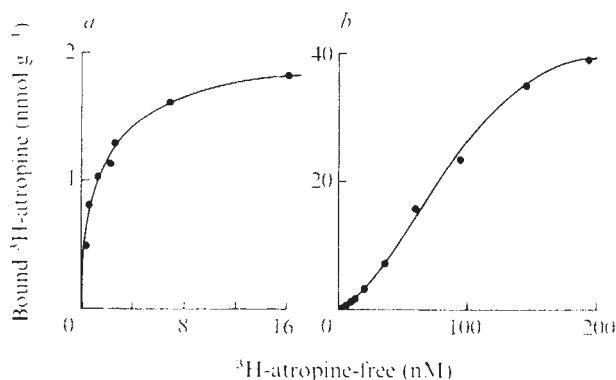


Fig. 1a, Binding of ^3H -atropine to a crude synaptosomal preparation from ox cerebral cortex. The subcellular fraction was the P_2 pellet of Gray and Whittaker¹⁴. A sample (0.25 ml) of the P_2 suspension in 30 mM sodium phosphate buffer containing 100 mM sodium chloride, pH 7.4, at a protein concentration of 0.5–1.5 mg ml⁻¹ was incubated at the room temperature for 10 min with ^3H -atropine with and without unlabelled atropine. A 200- μl sample was withdrawn and filtered on Millipore membranes and washed with 20 ml cold distilled water. Because the 'off' reaction of atropine from receptor is slow, the quantity of drug removed by this wash from the receptor should be very small. The filter was dried, then counted by liquid scintillation spectrometry in toluene-POPOP. The specific binding shown in the graph represents the difference in counts between uninhibited and inhibited (1 μM atropine) samples. $K_d = 2.0$ nM; $n_H = 1.0$. Apart from the binding site shown, two others were detected at higher concentrations of atropine, with $K_d = 4 \times 10^{-7}$ M and $K_d = 6 \times 10^{-6}$ M, their capacities being 20 and 180 nmol ^3H -atropine bound per g of protein respectively. These two sites were not susceptible to muscarinic agents and thus unlikely to be related to the muscarinic receptor. ($\pm$) ^3H -atropine (170 mCi mmol⁻¹) was purchased from the Radio Chemical Centre, Amersham. **b**, Binding of ^3H -atropine to the solubilised receptor. The solubilised material was prepared as follows. Equal volumes of P_2 suspension (30 ml, at a protein concentration of about 10 mg ml⁻¹) and 4 M NaCl were mixed—giving a final NaCl concentration of 2 M—and the suspension was stirred vigorously for 2–3 h at 4 °C. It was then centrifuged at 15,000 r.p.m. (28,000g) for 20 min, and the supernatant was diluted fourfold with 100 mM phosphate buffer pH 7.4 and recentrifuged at 25,000 r.p.m. (83,000g) for 2 h in the SW 27 rotor of a Beckman LS-40 ultracentrifuge. To the resulting supernatant was added ammonium sulphate (55 g per 100 ml, over a period of 1 h during which the medium was stirred constantly) and the material was then centrifuged at 15,000 r.p.m. for 20 min to obtain the precipitated proteins. The combined pellets were dissolved in 5 ml 100 mM phosphate buffer pH 7.4, and dialysed exhaustively to remove ammonium sulphate. Finally, the dialysate was centrifuged at 15,000 r.p.m. for 30 min and the supernatant (referred to as the solubilised material) was assayed for ^3H -atropine binding. About 5% of the total membrane proteins was solubilised. Proof that the material was soluble came from observations that no particulate matter was visible under the electron microscope and that the material did not sediment when centrifuged at 83,000g for 2 h. Binding of ^3H -atropine to the solubilised material was measured by rapid filtration on cellulose nitrate membranes (Selectron-Filter, type BA 85, 0.45 μm , Schleicher and Schüll). Free ^3H -atropine was removed by washing the membranes with 20 ml cold distilled water. We could detect no binding if the solubilised material was heated for 10 min at 60 °C before binding assay. $K_{1/2} = 80$ nM; $n_H = 1.5$. From comparisons of atropine binding to the soluble material and to the P_2 fraction, before and after salt-treatment, we found that the number of solubilised binding sites was equal to 50 $\pm$ 10% of the high-affinity P_2 sites (assuming quantitative absorption on to the cellulose nitrate membranes). Binding of ^3H -atropine to the solubilised material is being studied by equilibrium dialysis to determine the effectiveness of the cellulose nitrate membranes in retaining the soluble material. ^3H -atropine binding to the solubilised material was unaltered after storage at 4 °C for 5 d.

32% reported by Hulme *et al.*^{9,10}. These two components of the curve could represent identical muscarinic binding sites under different membrane restraints, or alternatively, only the high affinity site may be related to the receptor. A single binding constant was found for pilocarpine and arecoline (10^{-5} M and 2×10^{-5} M, respectively) and choline was ineffective up to millimolar concentrations. The K_i values found for both agonists and antagonists agree reasonably well with those reported by Yamamura *et al.*⁸ for subcellular brain preparations and with the agonist binding associated with smooth muscle contraction^{1,13}. Drugs unrelated to the muscarinic system generally did not displace the binding of labelled atropine.

After treatment of a crude synaptosomal preparation with 2M NaCl and separation of the solubilised material by centrifugation, binding of ^3H -atropine could be detected in the solubilised fraction by a filtration assay (Fig. 1b). The binding curve was sigmoidal ($n_H = 1.5$), half-saturation ($K_{1/2}$) occurring at 80 ± 30 nM, and the concentration of binding sites was 40 ± 20 nmol g⁻¹ protein.

The concentrations of unlabelled antagonists which reduce by 50% the level of specifically-bound ^3H -atropine (80 nM) (ID_{50} values) were found to be between 20 and 50 times higher than the corresponding concentrations found in synaptosomal preparations (Table 1). A single class of binding sites was found for each antagonist tested, the Hill coefficient being slightly greater than one (1.3–1.5). Competition by agonists of ^3H -atropine-binding was studied in the same way, as shown in Fig. 2 for acetylcholine. A single binding component was observed with $\text{ID}_{50} = 10^{-5}$ M: the Hill coefficient was 1.2. This competition was similar to the high affinity component observed for membrane preparations (Fig. 2). Oxotremorine, acetyl- β -methylcholine and carbamylcholine also displayed single binding constants (ID_{50} of 10^{-6} M, 10^{-5} M and 6×10^{-6} M, respectively).

The solubilised material thus contained a single, high affinity binding site for agonists with an ID_{50} value which closely paralleled that of the high-affinity component in the subcellular preparation. The low-affinity site was either lost, inactivated, left unsolubilised or converted to the higher affinity form. Antagonists bound to the soluble fraction with QNB being the most active, followed by scopolamine, atropine and lachesine—the same order as binding to the membrane fraction—but in a sigmoidal fashion and with lower affinities than to the membrane preparations. Such cooperativity may represent a transition of the soluble protein from a state of low to high affinity towards

Fig. 2 Inhibition of specific ^3H -atropine-binding to the P_2 fraction and to solubilised material by acetylcholine in the presence of 1 μM eserine sulphate. The concentration of the ^3H -atropine was 4 nM for the P_2 fraction and 80 nM for the soluble fraction. Binding was measured as described in the legend to Fig. 1. $\circ$, P_2 fraction; $\bullet$, P_2 fraction—high-affinity binding component; $\times$, solubilised material.

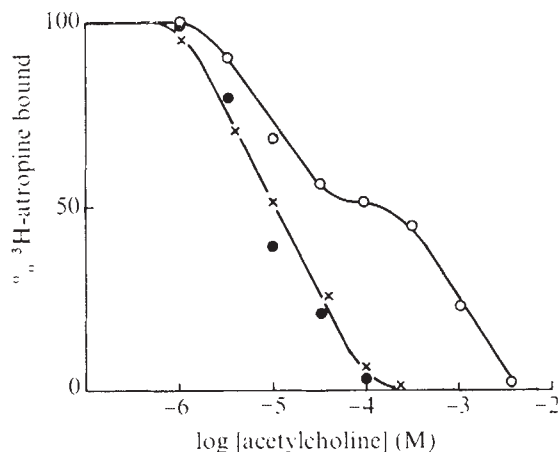


Table 1 Binding characteristics for membrane-bound and soluble atropine-binding material

Drugs	Rat brain* ID ₅₀ (M)	Particulate preparations Rat brain† ID ₅₀ or K _i (M)	Ox brain§ K _i (M)	Soluble material Ox brain§ ID ₅₀ (M)
(±) Atropine	10 ⁻⁹ –2 × 10 ⁻⁹ (8)	1.2 × 10 ⁻⁹ (11)	2 × 10 ⁻⁹	8 × 10 ⁻⁸
(±) Scopolamine	8–9 × 10 ⁻¹⁰ (8)	6 × 10 ⁻¹⁰ ‡	9 × 10 ⁻¹⁰	6 × 10 ⁻⁸
Lachesine		1.5 × 10 ⁻⁹ (11)	3 × 10 ⁻⁹	15 × 10 ⁻⁷
(±) QNB	4–5 × 10 ⁻¹⁰ (8)	2 × 10 ⁻¹⁰ (11)	2 × 10 ⁻¹⁰	4 × 10 ⁻⁹
Acetylcholine	2–4 × 10 ⁻⁶ (8)	3.3 × 10 ⁻⁷ (9, 10) 2.5 × 10 ⁻⁵ (9, 10)	3 × 10 ⁻⁶ 3 × 10 ⁻⁴	10 ⁻⁵
(±) Acetyl-β-methyl choline	3–5 × 10 ⁻⁶ (8)	8 × 10 ⁻⁷ ‡ 6.6 × 10 ⁻⁵ ‡	3 × 10 ⁻⁶ 10 ⁻⁴	10 ⁻⁵
Oxotremorine	5–8 × 10 ⁻⁷ (8)	4.6 × 10 ⁻⁷ ‡	3 × 10 ⁻⁷ 2 × 10 ⁻⁵	10 ⁻⁶
Carbamylcholine	2–3 × 10 ⁻⁵ (8)	5 × 10 ⁻⁷ (9, 10) 1.5 × 10 ⁻⁵ (11)	2 × 10 ⁻⁶ 2 × 10 ⁻⁴	6 × 10 ⁻⁶
Arecoline	1–3 × 10 ⁻⁶ (8)	10 ⁻⁷ ‡ 10 ⁻⁵ ‡	2 × 10 ⁻⁷	4 × 10 ⁻⁵
Pilocarpine	7 × 10 ⁻⁶ (8)	6.7 × 10 ⁻⁶ ‡	10 ⁻⁵	2 × 10 ⁻⁵

From competition curves with ³H-atropine, apparent inhibition constants were obtained for the membrane-bound material, according to the equation $K_i = ID_{50}/(1 + C/K_d)$ where C is the concentration of radioactive ligand and K_d its dissociation constant (the concentration of (±) ³H-atropine was 4–6 nM). In the case of the soluble preparation, ID₅₀ values are reported because of the complex nature of the atropine-binding curve (Fig. 2). (±) ³H-atropine concentration was 80 nM. The following compounds had no effect on binding of labelled atropine at 20 μM: choline, *d*-tubocurarine, gallamine, α -toxin from *Naja naja nigricollis*, decamethonium, eserine, neostigmine, edrophonium and Tetram (2,2-diethylamino)ethylphosphorothioic acid, 0,0-diethyl ester).

*All values are ID₅₀, that is the concentration of drugs which reduced ³H-QNB binding by 50% (concentration of ³H-QNB was 6 × 10⁻¹¹ M). Reference numbers are given in parentheses.

†Values given are either dissociation constants (obtained from the reciprocal of the quoted affinity constant (ref. 11 and personal communication from N. J. M. Birdsall)) or ID₅₀ values^{9,10}, all derived from ³H-propylbenzilylcholine displacement.

‡Personal communication from N. J. M. Birdsall.

§Results from the study reported here.

antagonists. Analogous changes in binding properties have been observed in other systems after solubilisation, notably in the case of the nicotinic receptor¹⁵. These results suggest that the solubilised material which shows specific atropine binding was derived from the high-affinity component of the membrane preparation which is most likely to be the muscarinic receptor site.

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Calcium levels in the brain underlie temperature control during exercise in the primate

ALTHOUGH it is known that the hyperthermia in man accompanying steady-state submaximal exercise is a regulated rise which is virtually independent of the ambient temperature¹, a fundamental question persists. What are the neural mechanisms involved in this regulated rise and in the ensuing hyperthermia encountered during

severe work in the heat? It has been proposed that the elevation in the temperature set-point associated with fever has as its basic component a change in the hypothalamic balance between the essential cations, Na and Ca²⁺ (refs 2, 3). Is it possible that such a mechanism could underlie the shift in temperature set-point which occurs during exercise? To explore this idea, we undertook two types of experiments. First, an artificial cerebrospinal fluid (CSF) containing excess Ca²⁺ ions was infused into the third ventricle. Secondly the kinetics of endogenous Ca²⁺, as reflected by its retention or release into the third ventricle, was analysed. We report here that Ca²⁺ ions, acting directly on the brain, can indeed attenuate or entirely block the hyperthermia of exercise without necessarily interfering with work output; further, the actual efflux of Ca²⁺ ions from the diencephalon increases markedly during the exercise-induced hyperthermia.

Macaque monkeys were used, as the heat-regulating processes in man and subhuman primates are similar⁴. Two monkeys were seated in primate chairs and trained to exercise strenuously on a 'rowing machine'⁵, using food pellets as reinforcers, so that they lifted up to three times their own body weight. Once the work rate had stabilised for an interval of 30 min. over several days, surgery was undertaken. Following neurosurgical procedures⁶ an array of three cannulae was implanted aseptically with the tips resting in the third ventricle; their patency was verified by the standard CSF pulsatile method. On recovery, the monkeys were exercised again for two 30-min periods every day for 2 weeks before infusions were undertaken.

When 0.5 ml of CSF was infused into the third ventricle at a constant rate over 10 min. the monkey continued to exercise, and its typical exercise-induced rise in colonic temperature persisted unabated. As shown in Table 1, however, the addition of excess Ca²⁺ ions to the infusion medium attenuated the hyperthermia in a dose-dependent manner. In one monkey (A), the elevation in colonic temperature was reduced by 72%, yet total work output was reduced by only 37%. In another monkey (B), the rise in its body temperature due to exercise was virtually blocked (+0.2 °C) after the infusion of excess Ca²⁺ ions, yet the decline in total work performed was less than 25%. In both cases, when Ca²⁺ was infused into the monkey's ventricle in a sufficient concentration to induce a hypothermia, the monkey nevertheless exercised to a substantial degree. On the basis of X radiographs taken in the sagittal plane, the differences in location of the tips of the cannulae may account for the differences in the animal's responses.

Table 1 Changes in colonic temperature from baseline control and total amount of work performed during exercise in neutral, cold and hot environments

Neutral (22 °C db)	Solution	Temperature change (°C)	Total work (J per 30 min)
Monkey A	Control 5-ion	+1.45	2018
	Ca ²⁺ 2.5 mM	+0.40	1258
	Ca ²⁺ 10 mM	-0.45	1018
Monkey B	Control 5-ion	+1.35	2795
	Ca ²⁺ 10 mM	+1.05	2556
	Ca ²⁺ 15 mM	+0.20	2181
	Ca ²⁺ 20 mM	-0.40	1045
Heat (35 °C db) Monkey A	Control 5-ion	+1.10	1338
	Ca ²⁺ 2.5 mM	+0.45	1192
Cold (0 °C db) Monkey A	Control 5-ion	+0.55	2036
	Ca ²⁺ 2.5 mM	+0.05	1367

To examine the effect of ambient temperature, we changed the environmental temperature of the monkey by a stream of hot or cold air blown into its chair 15 min before exercise and for the 30 min interval of exercise. In one monkey (A) we raised the ambient temperature to 35 °C and found that the excess calcium infusion (Table 1) greatly attenuated the exercise-induced rise in temperature without markedly affecting the amount of work performed. On the other hand, after the same monkey's ambient temperature was lowered to 0 °C (Table 1) on another day, its usual rise in temperature was abolished, but its work output was reduced by only 33%.

To determine whether a physiologically-induced change in endogenous calcium actually occurs within the diencephalon of the monkey as it exercises, we radiolabelled the structures surrounding the third ventricle by injecting 6.0–8.0 μ Ci of ⁴⁵Ca²⁺, in a volume of 6 μ l, directly into the third ventricle on the day before an exercise experiment began. Before the 30 min of intense exercise, a series of 5-min push-pull perfusions of the tagged region were carried out at 15-min intervals in order that a stable washout curve of ⁴⁵Ca²⁺ radioactivity could be generated. As soon as each monkey began to exercise, the efflux of ⁴⁵Ca²⁺ into the perfusate rose sharply from 100 to 300% (Table 2). At the same time, the typical exercise-induced rise of temperature occurred. Following

Table 2 Percentage increases in ⁴⁵Ca²⁺ activity above the baseline d.p.m. in perfusate collected during 30 min exercise (N=2) and in the 1 h post-exercise interval (N=3)

	During exercise	After exercise
Monkey A	163%	131%
Monkey B	249%	283%

the cessation of work, the augmented level of ⁴⁵Ca²⁺ in the perfusates (Table 2) continued for the next two to five perfusions, which corresponded to the interval of elevated body temperature.

Our experiments provide new evidence that Ca²⁺ is involved in the shift in temperature which occurs during exercise. In both the rat and dog exercising on a treadmill, excess Ca²⁺ ions infused intracranially also attenuate the usual rise in the animal's temperature^{8,9}. Therefore, it seems possible that the set-point for body temperature during exercise could be mediated by ions within the diencephalon², and that the set-point shifts upwards mainly by means of an unbinding, change in transport, or other activity of Ca²⁺.

It is clear that the rise in central body temperature during exercise⁹ can be modified or entirely abolished without necessarily incapacitating the animal or preventing it from performing a given work activity. These findings may have important implications for the clinical problems associated not only with the mechanism underlying heat stroke, but also with runaway hyperpyrexia.

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Chorionic membrane as an extra-adrenal source of foetal cortisol in human amniotic fluid

STUDIES in animals suggest that foetal cortisol (F) may have an important role in foetal lung maturation and the mechanism of parturition. In man the situation is complicated by the greater permeability of the placenta to this hormone¹ and its intraplacental conversion to its biologically inactive metabolite, cortisone (E) (refs 2–4). Many other foetal tissues also convert F to E (ref. 5). As a result, human foetal blood contains a relatively large amount of E, and a lower F:E ratio. Recently, we observed that the F:E ratio of amniotic fluid is considerably higher than that of cord serum⁶. To explore this, the possible influences of the foetal kidney, skin, gastro-intestinal tract, lung and membranes were investigated; only the chorionic membrane was found to convert cortisone to cortisol.

Foetal tissues and fluids were obtained at hysterotomy in patients undergoing abortions for maternal indications from 11–22 weeks gestation, at elective Caesarean section and vaginal delivery at term and from two anencephalic stillbirths. Placenta and membranes were also obtained at vaginal delivery and amniotic fluids at amniocentesis. Endogenous F and E concentrations in fluids and homogenised tissues were assayed after Sephadex LH-20 column chromatography, by radiotransassay using dog transcortin as described previously^{7,8}. For the enzyme studies, pieces of tissue (0.5–1 g) were weighed, minced (except for amnion and chorion which were left intact) and added to 2.0 ml medium (0.9% saline, amniotic fluid, Krebs-Ringer bicarbonate glucose buffer, or phosphate buffer, pH 7.4). Since similar results were obtained for all media, later studies used saline only. Incubations were carried out for 2 h at 37 °C in glass counting vials containing tracer amounts (100 pg) tritiated F or E with or without added cold steroid. Following addition of 10 ml ethyl acetate the vials were capped and shaken vigorously for 10 min. Organic phase 5 ml was evaporated to dryness under air, dissolved in 0.2 ml methylene chloride: methanol 98:2 and applied to Sephadex LH-20 columns⁸. This procedure separated the F and E fractions so that the radioactivity in each could be determined. Conversion was calculated as $100 \times \text{c.p.m. product} / \text{c.p.m. product} + \text{substrate}$, less the control value calculated in the same way. Recovery of radioactivity as E+F was 80–100% that of the controls which consisted of tracer in medium prepared and incubated along with the tissues. Alternative controls consisted of identical tissue preparations placed in a boiling water bath for 5 min before

Table 1 F and E in foetal fluids (ng ml⁻¹)

Gestational age (weeks)		n	Amniotic fluid			n	Cord arterial serum			n	Cord venous serum		
			F	E	F/E		F	E	F/E		F	E	F/E
11-16	Mean s.e.m.	11	3.0 0.6	9.2 1.6	0.33* 0.04	2	12.2 2.6	32.5 4.5	0.47 0.11	2	6.5 1.9	52.3 7.6	0.18 0.02
17-22	Mean s.e.m.	11	4.7 0.9	6.7 1.0	0.66*†‡ 0.07	6	6.7 2.6	17.3 4.5	0.35† 0.11	6	3.7 1.9	20.7 7.6	0.15‡ 0.02
25-30	Mean s.e.m.	4	9.4 1.7	5.1 1.1	1.8 0.70								
35 (anencephalic)	No. 1 No. 2	2	24.7 16.3	28.0 16.0	0.88 1.02	2	15.6 8.9	80.0 60.5	0.20 0.20	2	39.7 13.7	163 73	0.24 0.19
36-40 (no labour)	Mean s.e.m.	7	18.0 3.0	10.6 2.6	3.8 1.8	5	36.0 7.1	62.0 6.9	0.57 0.11	5	20.5 5.6	70.5 3.6	0.29 0.08
36-40 weeks (Spontaneous labour)	Mean s.e.m.	5	28.5 8.3	18.9 7.1	1.5 1.0	6	120 25	108 15	1.2 0.4	6	98 27	143 17	0.76 0.11

* $P < 0.001$; † $P < 0.05$; ‡ $P < 0.001$.

incubation. Identity of the products isolated from membranes and placenta was confirmed by recrystallisation to constant specific activity.

To simulate conditions *in vivo* more closely, four experiments were done in which layers of chorion and amnion from term placentas were placed over small shallow chambers 5 cm in diameter, tracer in 10 ml amniotic fluid was placed in the cavity lined by amnion, and the dishes were incubated for 2 h at 37 °C. Qualitatively similar though quantitatively about half the conversion seen in the routine incubation conditions was observed. When 10 ml saline was placed on the chorionic side, about one-third of the total activity passed through, mainly in the converted form. When the tracer was placed in the saline, about one-third passed into the amniotic fluid, again mainly as cortisol. These studies show that steroids can pass through the membranes from either the maternal or foetal side. It seems likely, however, that diffusion between uterus and chorion would be slow, whereas that between amnion (and chorion) and amniotic fluid would be hastened by the movement of the amniotic fluid itself.

Table 2 Interconversion of F and E in foetal tissues (%)

Tissue	Gestational age (weeks)	F → E	E → F
Kidney	12	39	3
	20	55	0
	35 (An)	83	3
Skin	12	24	4
	20	59	3
	35 (An)	17	2
Cord	20	3	2
GI tract			
	All	20	3
	Small bowel	27	17
	Stomach	12	1
	Small bowel	20	0
	Large bowel	1	2
Lung	12	54	3
	18	56	10
	35 (An)	22	3

An, Anencephalic.

Table 1 compares the endogenous concentrations of steroids found in cord serum and amniotic fluid. The F:E ratio was consistently higher in amniotic fluid than in cord serum at all gestational ages and rose with increasing age. F levels equalled or exceeded E levels in amniotic fluid from 25 weeks onward.

Table 2 shows the interconversion of F and E in several foetal tissues at various gestational ages. Conversion of F to E usually occurred while conversion of E to F was negligible, results in accord with previous studies⁵ and with

the endogenous concentrations shown in Table 3. The latter showed lower F:E ratios than serum at all ages studies.

In Fig. 1, the interconversions of F and E in chorion and in placental cotyledons are compared. The placenta showed high conversion of F to E throughout pregnancy. The reverse conversion occurred in the chorion with high values from 16.5 weeks until after labour had started, when they fell. The back reactions were consistently low averaging 65% for placental E→F and 10.2% for chorionic F→E.

Table 3 Endogenous F and E in foetal tissues (ng g⁻¹)

Tissue	Gestational age (weeks)	F	E	F:E
Kidney	12	2.2	24.0	0.09
	20	0.3	13.4	0.02
	35 (An)	4.1	61.4	0.02
Skin	35 (An)	0.3	10.7	0.03
GI tract				
	35 (An)	0.5	14.0	0.04
	Small bowel	0.7	21.3	0.03
	Large bowel	0.5	10.0	0.05
Lung	12	1.3	21.7	0.06
	20	1.3	16.8	0.08
	35 (An)	2.7	18.8	0.14

An, Anencephalic.

Conversion in both tissues was greatly decreased by boiling. No differences were seen in conversion in either direction when 100 ng ml⁻¹ F or E was added to the incubation medium of three placentas and three chorionic membranes (two from early pregnancy, one from late pregnancy). Little or no activity was seen in the amnion. When the chorionic membranes were examined microscopically, a layer of

Fig. 1 F-E interconversion: in placenta F→E (○) and chorion E→F (●). An, Anencephalic; dotted lines, mean trends.

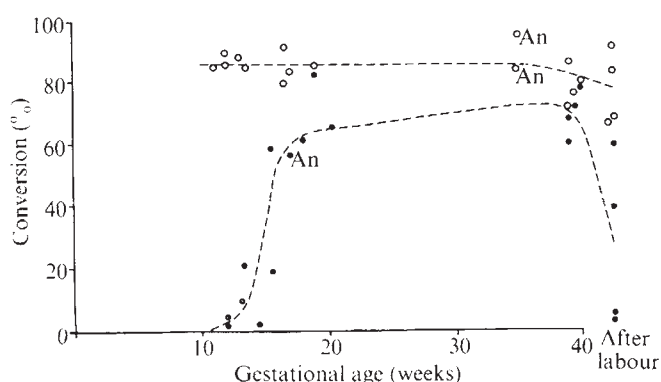


Table 4 Endogenous F and E concentrations in placenta and chorion (ng g⁻¹)

Gestational age	(Weeks)	Placenta			Chorion		
		F	E	F : E	F	E	F : E
Early pregnancy	13.5	2.0	183	0.012	13.7	25.0	0.55
	16.5	0.8	50	0.015	4.6	21.5	0.21
	16.5	3.0	117	0.026	69.5	2.0	34.7
	17	3.0	103	0.029	21.6	4.0	5.4
	19	2.5	60	0.042	28.0	0.6	44.8
Premature (anencephalic)	35	12.9	295	0.044	194	47.0	4.1
	35	5.4	160	0.034	54	19.2	2.8
Term	38+	—	—	—	11.4	0.6	18.0
	39	21.9	57	0.38	59.0	14.0	4.2
	40	7.5	489	0.015	16.3	4.0	4.1
	40	7.9	226	0.034	52.5	16.1	3.3
	40	6.7	253	0.027	43.5	11.5	3.8
Mean				0.06			12.5*
s.e.m.				±0.03			±4.8

*Excluding values from 12–16.5 weeks with F : E < 1.

maternal decidua was frequently adherent. Membranes from which this material was carefully scraped off retained their original activity. The uterine wall was also found to contain the same or a similar enzyme and this is currently under investigation⁹. No significant conversion of E or F was found when chorionic membrane from a single guinea pig killed under ether anaesthesia at 58 d gestation was incubated similarly.

The results of these *in vitro* studies are further substantiated by the endogenous levels shown in Table 4. A high F : E ratio was seen in chorion and a much lower ($P < 0.02$) one in placenta.

These observations show that the chorionic membrane is a metabolically active tissue which behaves differently from placental cotyledons. The rise in chorionic conversion of E to F in early pregnancy corresponds to the rise at 15–20 weeks in amniotic fluid cortisol which we reported previously¹⁰. This chorionic conversion of E to F accounts also for the rising F : E ratio in amniotic fluid relative to cord serum observed through pregnancy. The declining activity in labour accords with the lesser change in amniotic fluid F : E ratio during labour despite the surge of F in cord blood associated with its spontaneous onset.

These results may explain the poor correlation we have found between amniotic fluid cortisol and lecithin or the palmitic : stearic ratio (ref. 11). Amniotic fluid cortisol is mostly diffusible¹² and is presumably taken into the foetus by swallowing or other means, possibly by diffusion into the lung. In sheep the lung is known to produce fluid steadily, making absorption of cortisol unlikely¹³. In man there are few data and these are controversial¹⁴. Since the chorion in anencephalic infants seems to be normally active and since some of these infants breathe at birth, absorption of amniotic F through the lung or open cranium, might account for the relatively normal lung development seen in some anencephalics despite poor adrenal function¹⁵.

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Spectrin is absent in various tissue culture cells

A HIGHLY sensitive radioimmunoassay has been developed for human spectrin, one of the major components of the erythrocyte membrane^{1–6}. We report here use of this assay on cells of human, monkey and rat origin and show that spectrin is absent from all cell lines studied, with the exception of erythrocytes. These findings seem important when models which invoke spectrin to regulate membrane fluidity,^{7,8} or membrane-cytoskeletal interaction^{9,10} are considered.

Spectrin is extracted from erythrocyte ghosts by alkaline solutions of low ionic strength³. Purified spectrin consists of two high molecular weight polypeptides (molecular weights of 250,000 and 220,000) that are different from the myosin heavy chains^{2,3}. The properties that make spectrin of major interest for generalisation about a specific membrane protein are its abundance in the membrane protein fraction (approximately 25% of membrane protein)^{1–5} and its localisation exclusively on the cytoplasmic side of the erythrocyte membrane^{5,6}. In addition, it is generally assumed that spectrin has an important role in regulating the mobility of transmembrane particles in the plane of the membrane^{7,8} and that an interaction between spectrin and actin is involved in maintaining the shape and morphology of the erythrocyte^{9,10}. Particularly interesting in this respect are reports on the association of spectrin and actin and the effects of spectrin on the *in vitro* polymerisation of actin^{11,12}. Proteins with 'spectrin-like' properties (that is, high polypeptide molecular weight, solubility in low salt, and lack of myosin-like ATPase activity) have been found in echinoderm sperm¹³, sea urchin eggs¹⁴, macrophages¹⁵ and *Acanthamoeba*¹⁶ where they are also assumed to regulate the polymerisation of actin. These studies suggest that spectrin may be an ubiquitous protein in eukaryotic cells. Painter *et al.*¹⁰, however, were unable to detect spectrin by complement fixation in HeLa cells or in human fibroblasts, lymphocytes or platelets at a level higher than the background of the assay which was approximately 0.1% of the total cell protein. This assay was not very sensitive because even in erythrocytes spectrin accounts for only approximately 0.3% of the total cell protein⁵ and because a 'spectrin-like' actin-binding protein has been found at the 1% level¹⁵. Concentrations of spectrin corresponding to 5% of the myosin content of most non-muscle cells would not have been detected, so the data of Painter *et al.*¹⁰ do not exclude a potential

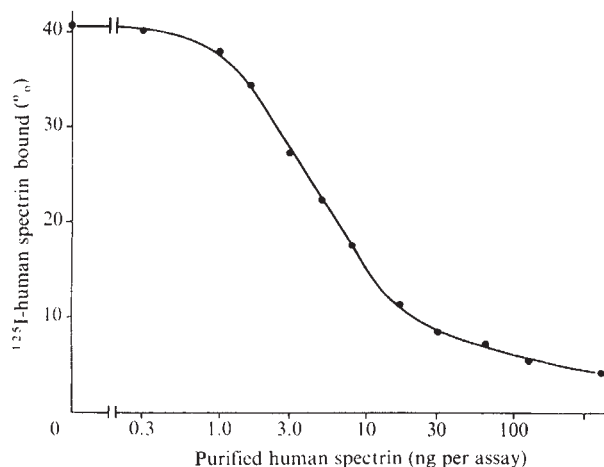


Fig. 1 Competition of unlabelled with ^{125}I -labelled human spectrin for binding to a specific antibody. Purified spectrin was iodinated in a reaction mixture (60 μl) to which 2 μg human spectrin, 0.5 mCi carrier-free $\text{Na } ^{125}\text{I}$, 25 μg Chloramine T, and 62 μg $\text{Na}_2\text{S}_2\text{O}_5$ were sequentially added¹⁸. After separation of the labelled protein and unreacted $\text{Na } ^{125}\text{I}$ by gel filtration on Sephadex G-25, the radioactively labelled spectrin was rechromatographed on Sepharose 4B in order to eliminate some aggregated material. The specific activities of the preparations varied between 45 and 100 μCi per μg of spectrin. Unlabelled human spectrin (0.2 ml) at various concentrations was preincubated (18 h at 4°C) with 0.1 ml of a crude IgG fraction (21.4 mg protein per ml; diluted 8000-fold) of rabbit anti-human spectrin. After addition of 0.1 ml ^{125}I -spectrin, equivalent to about 0.2 ng of protein, incubation was continued for 48 h. Carrier rabbit IgG and enough sheep anti-rabbit IgGs were added to precipitate the formed antigen-antibody complexes maximally (24 h at 4°C). Labelled spectrin and the various IgG-dilutions were kept in buffer containing 50 mM Na-phosphate, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1 mM phenylmethane sulphonyl fluoride (PMSF), 0.02% Na_3A and 0.2% bovine serum albumin (BSA). Unlabelled spectrin was diluted with 30 mM Tris-HCl, pH 8.0, 1% Triton X-100, 0.1 mM EDTA, 0.1 mM PMSF and 0.2% BSA. The protein concentration of pure spectrin was determined using the extinction coefficient ϵ 280 nm = $1.01 \text{ ml cm}^{-1} \text{ in mg}^{-1}$ (ref. 2).

function of spectrin as a regulatory protein of the microfilament system.

In order to increase the sensitivity of a spectrin-specific assay we have developed an immunological competition assay in which unlabelled spectrin competes with highly radioactively labelled spectrin for binding to a limited amount of spectrin-specific antibody (Fig. 1). Spectrin was purified from human erythrocyte

ghosts by standard procedures¹⁷. Gel filtration on Sepharose 4B removed not only impurities but also erythrocyte actin. The resulting preparation showed only the two high molecular weight polypeptides even on heavily overloaded polyacrylamide gels containing sodium dodecylsulphate. Antibodies against native spectrin were raised in rabbits. The antisera formed a single precipitin band on immunodiffusion against human spectrin.

The radioimmunoassay (Fig. 1), which to our knowledge is the first one for spectrin, can detect as little as 5 ng of erythrocyte spectrin per ml. Thus in extracts of human cells this assay should detect spectrin at levels as low as 10^{-4} – $10^{-3}\%$ of total protein.

Table 1 summarises our results on the radioimmunological detection of spectrin in extracts of various tissue culture cells of human and monkey origin. Spectrin could not be detected antigenically in any of the cell lines studied, including both fibroblasts and epithelial cells. HeLa cells, grown either in suspension or in monolayer were devoid of spectrin, in spite of the differences in morphology and cell shape present in the different growth conditions. In these cells the assay would detect spectrin-cross-reacting material if there were more than approximately 250 molecules of spectrin in a single cell. Spectrin added exogenously to the cell homogenates was easily detected in the radioimmunoassay and the estimated values were in good agreement with the known input (see Table 1). Thus potential loss of spectrin due to proteolytic degradation during the long incubation time used can be ruled out. For intact erythrocytes Bretscher⁵ reported a ratio of 45 mg haemoglobin to 0.6 mg membrane proteins; assuming about 25% of the mass of the membrane proteins to be spectrin leads to a value $< 0.32\%$ of the total erythrocyte protein being spectrin. Our estimation (0.24%) is in good agreement with this calculation, suggesting that in the conditions used spectrin is almost completely extracted from the cells.

In contrast to an earlier antibody preparation²⁰, our rabbit anti-human spectrin sera showed a weak but reproducible precipitation with spectrins purified from sheep or rat erythrocytes. This cross reactivity allowed use to use the anti-human spectrin sera also for the estimation of spectrin in different tissue culture cells of rat origin. In this system the heterologous antisera must be fivefold more concentrated to give comparable binding. The sensitivity in these conditions allows the estimation of about 50–100 ng of rat spectrin per ml. In the homogenates of four different rat cell lines studied (2 cloned lines of SV 40 transformed embryo fibroblasts, the glial cell clone 6, and a line derived from a mammary

Table 1 Reaction of crude cell extracts and homogeneous human spectrin in a radioimmunoassay for spectrin

Disrupted cells and additions	Protein concentration of the assayed extract (mg ml^{-1})	^{125}I -spectrin (human) bound (% of input)	Amount of spectrin potentially present (% of extracted protein)
None	—	40.5	—
Human			
Erythrocytes	1.6×10^{-2}	18.3	2.4×10^{-1}
HeLa*	10.0	38.5	$< 4 \times 10^{-5}$
HeLa†	13.5	36.4	$< 4 \times 10^{-5}$
WI 38	9.4	38.8	$< 4 \times 10^{-5}$
SV 80	9.1	37.0	$< 5 \times 10^{-5}$
Spectrin	2.5×10^{-5}	22.5	100
HeLa† and Spectrin	13.5 and 2.5×10^{-5}	23.8	
Monkey			
Vero	9.8	40.1	$< 1 \times 10^{-5}$
CV 1	9.8	36.3	$< 6 \times 10^{-5}$

Washed cells were disrupted at ice-bath temperature by 30 strokes in a tight fitting homogeniser. The buffer used contained 30 mM Tris-HCl, pH 8.0, 1% Triton X-100, 0.1 mM EDTA and 0.1 mM PMSF. After 30 min on ice, unsolubilised material was removed by centrifugation (20,000g, 15 min, 4°C) and 0.2-ml aliquots of the supernatants were tested, in parallel with purified spectrin as standard in conditions as described in Fig. 1. Solubilisation of erythrocytes was almost complete. Examination of the small sediment by gel electrophoresis in SDS showed only traces of the characteristic spectrin doublet indicating that much less than 10% of the spectrin remained unextracted. This was also indicated by the results of the radioimmunoassay (see text). Protein was assayed by a modification of Lowry's procedure¹⁹.

* Grown as monolayer.

† Grown in suspension.

adenocarcinoma) no spectrin could be detected at a level higher than $3 \times 10^{-3}\%$ of the extracted protein.

In summary, it seems that erythrocyte-type spectrin is not an ubiquitous protein, although we can not exclude the unlikely possibility that non-erythrocyte cells contain spectrin(s) which are immunologically unrelated to erythrocyte spectrin. Spectrin is probably an important protein of the erythrocyte—and perhaps some yet unidentified cells—and probably has an important role for the special membrane of this highly differentiated cell.

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Precocious development of foetal glucuronidating enzymes induced by glucocorticoids in culture and *in utero*

CONJUGATION with glucuronic acid is the major pathway in vertebrates by which harmful organic compounds are detoxicated. It ensures the excretion of excess endogenous compounds such as steroid hormones, catecholamines and bilirubin, and of a great range of toxic xenobiotics such as drugs, pesticides, industrial pollutants and their metabolites¹. Glucuronidation, however, is virtually absent in the early mammalian foetus and approaches adult levels only in the perinatal period, putting the foetus and newborn at considerable risk from unwise medication or exposure to environmental contaminants. This susceptibility has been demonstrated frequently, often with fatal results^{2,3}. Defective glucuronidation of endogenous bilirubin is a major cause of human neonatal jaundice and can lead to infant death⁴. Attempts have been made to induce the responsible microsomal enzyme(s), UDP-glucuronyltransferase (EC 2.4.1.17), in cases of prematurity, genetically defective enzyme formation or poisoning by glucuronidogenic compounds; administration of xenobiotic inducers such as phenobarbital has had limited success in neonates and the term foetus²⁻⁵. Adequate treatment of such conditions, however, requires knowledge of endogenous compounds which induce the enzyme perinatally. We report here the first evidence in foetal mammalian tissue of precocious development of UDP-glucuronyltransferase activity and of overall glucuronidation brought about by known compounds of endogenous nature. These findings provide insight into the perinatal development of this important enzyme.

We have already established that glucocorticoids invoke precocious development of the transferase in chick embryo liver. As that work involved pituitary grafting and continuous infusion of hormone *in ovo*, its extension to the mammalian

foetus required simpler techniques. Operative stress to the mother, resulting in excessive corticosteroid production, would make controls difficult. We have therefore adopted two approaches: exposing the foetal liver to glucocorticoids in culture and *in utero* by simple maternal injection.

Culture methods were as described previously⁶. Exposure *in utero* was achieved by injecting pregnant rats intraperitoneally on days 14 and 15 of gestation with the steroid dissolved in arachis oil; control pregnant rats received arachis oil only. UDP-glucuronyltransferase activity towards *o*-aminophenol was assayed as described previously⁶ but, because the mammalian liver enzyme is markedly activable and the activation is age dependent, assays were carried out in each experiment over a range of digitonin concentrations (0–0.2% w/v) covering zero to beyond optimal activation. Overall glucuronidation, for which the intact cells synthesise their own UDP-glucuronic acid¹, was measured in sliced tissue, as previously⁶.

We found transferase activity towards *o*-aminophenol in the naturally developing rat foetus to be virtually zero from days 14–17 of gestation, increasing sharply on day 17 to peak on day 20. This held for all degrees of activation of the enzyme studied. We then exposed 14-d rat foetal liver explants to 2 μ M corticosterone or cortisol in culture in a protein-free defined medium. After 3 d in culture transferase activities had reached values normally found in 18–19-d foetal liver *in utero*, almost the 'adult' levels. Dexamethasone, 2 μ M in the culture medium, increased the values to full adult male levels. 'Control' 14-d foetal livers, left for 3 d *in utero*, possessed negligible levels, as did those cultured without the added glucocorticoid (Table 1).

Pulsing the glucocorticoid-exposed cultures for 5 h with

Table 1 Development of UDP-glucuronyltransferase activity in foetal rat liver exposed to glucocorticoids

Treatment of 14-d foetal liver sample	nmol <i>o</i> -aminophenyl glucuronide formed per mg protein per h	
	In culture	<i>In utero</i>
Fresh (untreated)	—	<2.0
After 3-d control treatment	2.8, 2.2	2.4, 2.7
After 3-d cortisol treatment	25.8, 25.0	7.0, 7.6
After 3-d dexamethasone treatment	45.3 $\pm$ 9.8 (4)	28.4, 30.8

Liver fragments were cultured on rafts over Eagles' MEM supplemented with L-glutamine and penicillin–streptomycin mixture⁶ and containing 2 μ M steroid where required. Pregnant rats were injected intraperitoneally with 8 mg cortisol or 0.3 mg dexamethasone in arachis oil per day on the days 14 and 15 of gestation. Control rats received arachis oil only; liver transferase levels in control foetuses were identical with those in foetuses from uninjected mothers. Adult male rat transferase levels were 30–40 of above units. Transferase activities were assayed⁶ in 'optimal' activation conditions (see text). Results above are from typical experiments. Each result is from a pool of at least 4 foetal livers; where appropriate, s.e.m. is given, with number of experiments in parentheses.

3.5 μ M cycloheximide inhibited ¹⁴C-leucine uptake and prevented development of transferase activity; both processes returned within 2 d to the rates observed in non-pulsed cultures. The data also indicated that general protein synthesis proceeded at similar rates in cultured liver whether exposed to glucocorticoids or not; further, after culturing for 2 d without glucocorticoids, subsequent exposure to them evoked transferase production as rapidly as in fresh cultures. The glucocorticoids were thus not needed to maintain viability of the cultured tissue but seemed responsible for the specific increase in transferase. Other steroids (not glucocorticoids), exhibited no effect.

Table 1 also shows the effect on foetal liver transferase of maternal injection of glucocorticoids. As glucocorticoids cross the rat placenta⁷, and dexamethasone is less likely to be inactivated by the mother⁸, the precocious development of transferase activity following injection of cortisol or dexamethasone

thasone presumably reflects the action of these compounds on the foetal liver *in utero* as described above during culture. Transferase stimulation was found to be linearly dose dependent.

Overall glucuronidation, as measured in foetal liver slices or explants, also precociously increased after exposure to the glucocorticoids in culture or *in utero*. We have induced precocious transferase activity to several substrates, endogenous and xenobiotic, this way but stimulation towards others, apparently those developing later in the perinatal period, have so far resisted our efforts both in culture and *in utero*.

We have thus reported the first evidence in foetal mammalian tissue of precocious development of UDP-glucuronyltransferase activity and of overall glucuronidation brought about by known compounds of endogenous nature. These compounds are glucocorticoids. Their effect is reproduced by the long-acting synthetic glucocorticoid, dexamethasone. They evoke high transferase activity in foetal rat liver from negligible levels, both in culture and *in utero*. They act directly on the liver, and require protein synthesis for their precocious induction of the transferase. Their involvement perinatally is consistent with the peak in corticosterone levels observed in foetal rat plasma on gestational day 19 and with the maternal increase on day 18 (refs. 9–11). We therefore suggest that the natural perinatal development of glucuronidation and UDP-glucuronyltransferase activity towards certain substrates is brought about by glucocorticoids.

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Liver protein metabolism response to cold in genetically obese (*ob/ob*) mice

THE response of obese (*ob/ob*) mice to the cold is reportedly impaired to the extent that they die 3–6 h after exposure. They also increase their oxygen consumption to a lesser extent than do their lean littermates in response to the cold^{1,2}. As *ob/ob* mice have increased insulation due to excess body fat and respond in this way to the cold before the phenotypic expression of obesity, impaired thermogenesis seems to be a primary feature of this strain of genetic obesity. Adult obese Zucker rats also show impaired thermogenesis when exposed to cold and their mean survival time is 28 h (ref. 2). The nature and control of this thermogenesis and its possible relevance to energy balance is unclear. The involvement of protein metabolism and the energy cost of protein turnover has been postulated by^{3–6}. Acclimatisation to the cold increases protein turnover in rats⁶ and in lean (*Ob/+*) mice (unpublished observations).

We report here a comparison of rates of amino acid

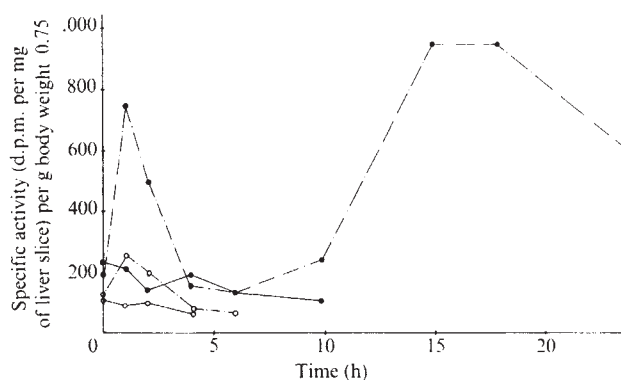
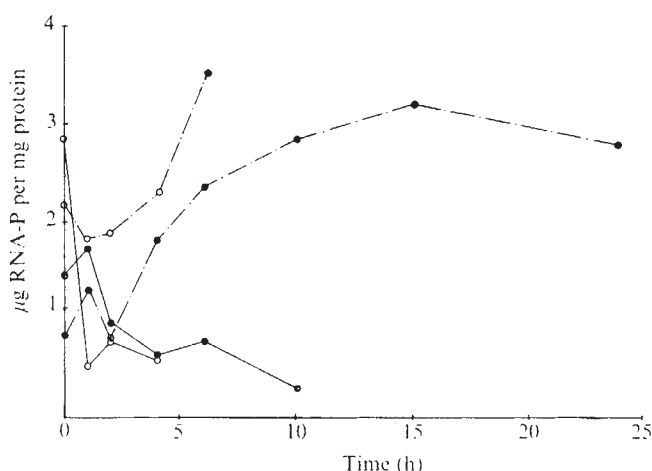


Fig. 1 Changes in liver amino acid incorporation following exposure to 4 °C in lean and obese mice with and without food. ○—○, obese without food; ○—○, obese with food; ●—●, lean without food; ●—●, lean with food. Animals were killed by cervical dislocation, the livers rapidly removed, placed on a Petri dish packed with ice and three or four slices approximately 1 mm thick were cut with a razor blade. The slices from each liver were placed in individual flasks with 2.5 ml of Krebs phosphate Ringer solution (100 parts 0.9% NaCl; 4 parts 1.15% KCl; 3 parts 1.22% CaCl₂; 1 part 3.82% MgSO₄ 7 H₂O and 20 parts 0.1 M phosphate (Na) buffer pH 7.4). The buffer was prepared from 0.1 M NaH₂PO₄·2H₂O (14%) and 0.1 M Na₂HPO₄·2H₂O (86%). 1 μCi of L-4,5-³H-leucine (55 Ci mmol⁻¹) was added, and after oxygenation the slices were incubated at 37 °C for 1 h. The incubation was stopped by removal of the slices, which were blotted and homogenised in TCA (100 g l⁻¹). The precipitate was washed twice with a saturated solution of unlabelled leucine in TCA (100 g l⁻¹) to remove any unincorporated labelled leucine. TCA was removed by washing with ether and the precipitate solubilised in 3 ml of 0.3 M KOH. The activity of the protein was measured by liquid scintillation counting and protein determination was by Autoanalyser*.

incorporation into liver protein by lean (*Ob/+*) and obese (*ob/ob*) mice. Amino acid incorporation was depressed in the obese mice, in response to a cold environment when compared to their lean littermates.

Female lean and obese mice, age-matched (70 ± 5 d) were kept at 22 °C and fed freely until required. Animals were then placed in a 4 °C environment with access to food, and killed in groups of four, from zero time to 24 h after cold exposure. The length of the comparative study was

Fig. 2 Liver RNA to protein changes following exposure to 4 °C in lean and obese mice with and without food. ○—○, Obese without food; ●—●, lean without food; ○—○, obese with food; and ●—●, lean with food.



controlled by the survival time of the obese (*ob/ob*). The livers were immediately removed, and amino acid incorporation into protein was measured as described in Fig. 1. The rest of the liver was used for RNA determinations⁷ and the residual protein pellet was solubilised in 5 ml of 0.3 M KOH for protein determination by Autoanalyser⁸.

Ratios of RNA to protein in livers from obese mice were significantly greater ($P < 0.001$) than those of lean mice at time zero. This was confirmed by monitoring the response to cold without food (Fig. 2). This suggests that there is some restriction on the effective use of RNA species after the transcription stage of protein synthesis in obese mice.

The ratio of RNA to protein was significantly greater in both lean and obese animals after exposure to cold (Fig. 2). But after 4 h, only the ratio for the lean animals was significantly greater ($P < 0.02$) than the corresponding ratio measured at time zero.

Amino acid incorporation in the lean mice was significantly increased after 1 h of exposure and the percentage increase over zero values was greater in the lean than the obese mice, whether expressed directly as specific activity of liver slice protein or as (Fig. 1) specific activity per unit of body wt^{0.75}. The results are expressed as the latter because if protein turnover is contributing to thermogenesis, the ability of the heat produced by the process—to maintain body temperature—must be related to metabolic body size (body weight^{0.75}). Obese mice were dead within 8 h and so a second increase in amino acid incorporation, like that found in the lean mice, could not be demonstrated. Either the failure of some other process caused a decrease in body temperature so that the suppression of amino acid incorporation was a secondary effect, or the increase of amino acid incorporation is critical to the maintenance of body temperature. The latter seems more likely as there is a significant difference between lean and obese mice with food and between lean without food after 1 h of cold exposure, when body temperature will not have fallen sufficiently to explain the decrease in amino acid incorporation⁷.

The secondary increase in incorporation in the lean

animals can be explained by the increase in RNA, whereas the primary increase in incorporation must be controlled by other factors, probably hormonal. But even though RNA levels in the obese mice after 6 h were significantly increased ($P < 0.001$) (Fig. 2) there was no associated increase in amino acid incorporation.

Unfed mice were used to find whether the increased food intake, by cold adapted animals⁹ and specifically by lean mice (unpublished observations) was essential for adaptation to the cold. In these circumstances neither lean nor obese mice could adapt to the cold, the obese mice died 2 h earlier than in the previous study and the lean animals died after 11 h of exposure. In neither lean nor obese mice was there an increase in amino acid incorporation and the RNA to protein ratio decreased in both groups (Figs 1 and 2).

These observations seem to support the hypothesis that food has a thermic effect¹⁰. But in a study performed at 22 °C when lean and obese animals were starved for 48 h and fed (Figs 3 and 4) there was only a twofold increase in amino acid incorporation, compared with the fivefold increase observed in the cold. We therefore suggest that

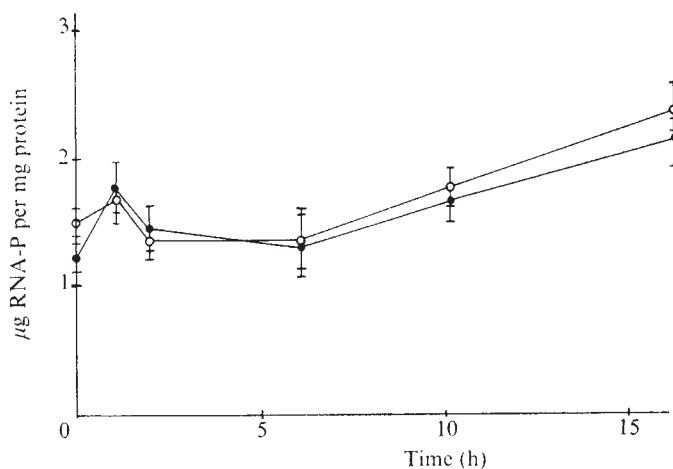
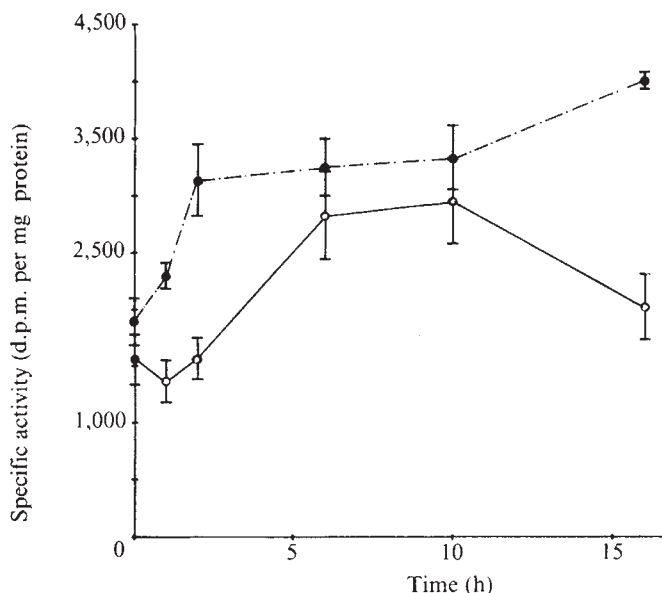


Fig. 4 Liver RNA to protein changes following a meal in lean and obese mice. ○, Obese; ●, lean.

Fig. 3 Changes in liver amino acid incorporation following a meal in lean and obese mice. ○ — ○, Obese; ● — — ●, lean. Animals were starved for 48 h, and then fed Porton mouse diet (Christopher Hill) for the remainder of the experiment. All animals after 1 h had full stomachs. Amino acid incorporation was measured as in Fig. 2.



food contributes directly to thermogenesis, but that the large increase observed in the cold cannot be explained entirely in this way. There must be simultaneous hormonal stimulus of thermogenesis and amino acid incorporation. No hormonal stimulus was observed in the cold without food presumably because substrate availability was rate limiting.

RNA to protein ratios for lean and obese mice were not significantly different for the starved animals, and were only slightly increased after feeding (Fig. 4). The levels in obese mice had decreased to those of lean mice after 48 h of starvation, which agrees with the observation that the former levels decreased to the latter values if mice were exposed to the cold without food. This supports the argument that RNA levels are high in the livers of obese mice because of an impaired protein synthesis. In starvation when protein synthesis is severely restricted due to lack of substrate, the effect of such an impairment which increases RNA would be negated.

In an obese animal placed in the cold without food, shivering thermogenesis would make such a major demand on substrate that protein synthesis would be restricted in a similar manner. Amino acid incorporation in the obese in response to food (Fig. 3) was depressed in comparison with the lean and was not so prolonged, which may affect

energy balance by depressing energy expenditure thus contributing to the obesity.

The difference between amino acid incorporation in the livers of lean and obese mice is not the only consequence of exposure to the cold. Jansky¹¹ has shown in cold-adapted rats that the liver only contributes 25% of non-shivering thermogenesis (NST), the remainder coming from muscle (50%), digestive tract (10%), brown fat (10%) and other tissues (5%).

It is not known whether protein metabolism in these tissues can be implicated in NST. Also, the involvement of futile cycles in thermogenesis has been postulated¹². Specifically, James and Trayhurn¹³ have implicated a futile cycle in glucose metabolism found in skeletal muscle, the impairment of which in obese subjects reduces their ability to produce heat, either in a cold environment or after food. They state that¹³, "The control and interrelationships between different futile cycles are at present unclear".

Bray *et al.*¹⁴ demonstrated that exposure to the cold does not lower the body temperature of obese (*ob/ob*) mice if they are pretreated with triiodothyronine, and that the body temperature of lean mice decreases if they have been made hypothyroid by prefeeding with propylthiouracil. This suggests thyroid hormones as likely candidates for the hormonal control. Jansky¹¹ demonstrated that noradrenaline also stimulates thermogenesis, so there are at least two candidates for hormonal control. The relative importance of each is unclear especially as the calorogenic effect of noradrenaline depends on thyroid status¹⁵.

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Contractile activation in slow and twitch muscle fibres of the frog

VERTEBRATE slow and twitch skeletal muscle fibres differ qualitatively in many respects and constitute functionally distinct motor systems^{1,2}. Little is known, though, of the respective contractile activation properties. Since triad contacts between transverse (T) tubules and sarcoplasmic reticulum (SR), thought to be the pathway for activation of twitch fibres, are nearly identical in frog twitch and slow fibres³, similar processes would be expected to lead to

Ca²⁺ release from the SR following T-tubule depolarisation in both muscle types. We describe here voltage-clamp studies of activating threshold contractions in slow and twitch fibres^{4,5}. Activation kinetics in these physiologically very different fibre types are remarkably similar. Thus, a given membrane potential change may result in an equivalent amount of Ca²⁺ delivered to both slow and twitch myofibrils. Once activated, each fibre type would shorten at its characteristic velocity⁶, probably determined by the rate of actomyosin cross-bridge turnover⁷.

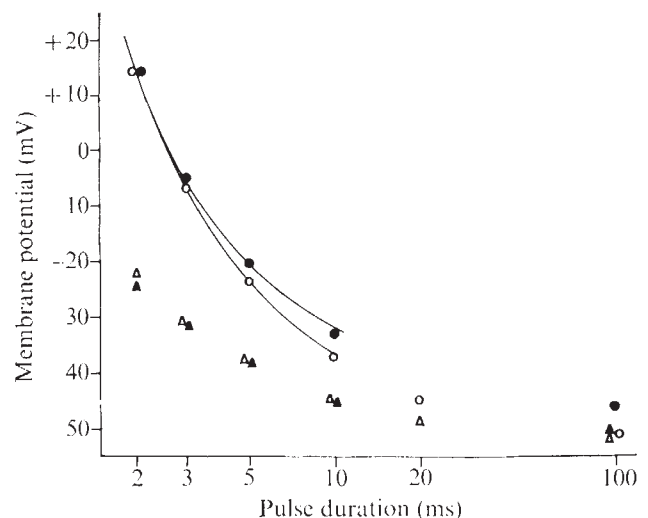
Experiments were performed at 9 °C on sartorius twitch fibres and pyramidalis slow fibres of English frogs (*Rana temporaria*). Normal Ringer contained 115 mM NaCl, 2.5 mM KCl, and 1.8 mM CaCl₂ buffered at pH 7.0 with 1.5 mM Na-phosphate; twitch fibres studied were poisoned with tetrodotoxin (10 µg ml⁻¹).

Generally we used the methods described by Costantin⁸. Membrane potential was measured differentially between an internal microelectrode and another 20–30 µm away outside the fibre. Time constants of these electrodes and command pulses to the clamp circuit were matched (~150 µs), and current-passing microelectrodes were shielded with conducting paint and then insulated. These procedures facilitated rapid membrane potential changes using high resistance electrodes necessary for the slow fibre experiments⁸. Holding potential was –90 mV throughout.

For a given pulse duration, the threshold membrane potential was taken as that at which discrete sarcomere shortening was visually detected, the region under observation being between the microelectrodes and in the plane of focus through the upper or lower surface of the fibre. For the briefest pulses, a range of up to 5 mV was occasionally required to decide on an unequivocal contraction; threshold was taken as the midpoint of such a span. This was more troublesome in slow fibres due to the low shortening velocity.

In a frog twitch fibre the amplitude of a step depolarisation eliciting a threshold contraction increases markedly as

Fig. 1 Strength–duration curves for twitch (●, ▲) and slow (○, △) muscle fibres. Circles represent means from 14 slow and 18 twitch fibres studied at 9 °C; triangles are from 21 slow and 18 twitch fibres at 20 °C. Smooth curves were calculated with the equation⁴: $(V_T + C)t_c = B_T$, where V_T is threshold membrane potential (mV), t_c is pulse duration (ms), and C and B_T are empirical constants determined as described by Costantin⁸. $B_T = 127 \pm 4.7$ mV.ms (s.e.m.) and $C = 49.1 \pm 0.9$ mV for slow fibres; $B_T = 116 \pm 5.6$ mV.ms and $C = 43.5 \pm 1.0$ mV for twitch.



pulse duration decreases, resulting in a familiar smooth strength-duration (S-D) relation^{4,5}. Such S-D curves for slow and twitch fibres in normal Ringers are shown in Fig. 1. Clearly, at either temperature, the relations for slow and twitch fibres are of the same form. At 9 °C the curves superimpose at short times and diverge to near-rheobase 100-ms values of -51 ± 0.8 mV (s.e.m.) in slow fibres and -46 ± 0.8 mV in twitch. At room temperature the curves for both fibre types are indistinguishable.

Although the S-D curve reflects events leading to Ca^{2+} activation of thin filaments, it is not clear which processes determine its exact form. During a long pulse, the situation is complicated, since both release and reuptake of Ca^{2+} by the SR will occur, and rheobase represents the steady-state with one process just balancing the other. Uptake of Ca^{2+} during a short pulse and for a few ms afterwards should be negligible⁹, so that only processes leading to Ca^{2+} release should fix the requirements a brief depolarisation must satisfy to activate contraction.

One starting point, then, for interpreting the comparison of S-D relations is to assume that for short pulses, a threshold contraction results from release of a particular amount of Ca^{2+} . Rate limiting steps leading to release could occur at the triad, presumably where T-tubule membrane potential is sensed by the SR, or at the sites of Ca^{2+} release themselves. Thus, 2 and 3 ms thresholds at 9 °C should be most indicative of gross differences in the activation kinetics in slow and twitch fibres, but such differences are not seen.

Another similar experiment involves studying the temporal summation of two sub-threshold depolarisations in activating a contraction^{4,5}. This yields information on the time course of decay in 'mechanical excitability' induced by the first pulse. The fraction of excitability remaining at any time after the first pulse can be estimated by comparing amplitudes of a second test pulse (V_2) eliciting a threshold contraction and of a single threshold pulse of identical duration (V_T ; see legend and insert in Fig. 2). To maximise the voltage range covered by V_2 , brief first pulses (80–90% of threshold) were followed with test pulses of identical duration (3 ms in twitch; 5 ms in slow).

Results from one twitch and two slow fibres are shown in Fig. 2. In all fibres the level of mechanical excitability decayed rapidly after the first pulse with a half-time of about 1 ms (Fig. 2a). All fibres then showed a slower phase of decay, but with vastly different rates in slow and twitch. Twitch fibre excitability decayed with a 5–6-ms time constant, threshold returning to the original V_T value within about 20 ms, whereas the excitability of the slow fibres returned to the normal level with a time constant of more than 300 ms (Fig. 2b). No such very slow component was seen in three other twitch fibres studied.

As argued by Costantin⁹, the fast initial decay in a twitch fibre probably represents rapid voltage-dependent deactivation of Ca^{2+} release following repolarisation. Since kinetics of mechanical activation, as evidenced by the S-D curves, seem indistinguishable in the two fibre types, the fast decay of excitability should also be alike in both, and this seems to be so.

Most striking are differences in the slow phase of excitability decay. For twitch fibres, this decay is of the proper time course to represent background Ca^{2+} removal by the SR^{5,10}. Since a slow fibre has less SR than a twitch fibre¹¹, the net rate of Ca^{2+} removal may also be lower, and the very slow decay might then also represent Ca^{2+} uptake. Preliminary experiments measuring tension produced by voltage-clamped short cruralis¹² slow fibres support this idea. After long (1–3 s), just suprathreshold depolarisations, relaxation proceeded with a half time of 0.5–1.0 s.

From these results it seems that the kinetics of mechanical

activation in frog slow and twitch fibres are quite alike. Furthermore, buffering the external Ca^{2+} concentration at $< 10^{-8}$ M with 2.5 mM EGTA and 10 mM MgCl_2 leaves the S-D curves of both fibre types essentially unchanged¹³, again suggesting a common mode of activation through release of Ca^{2+} by the SR.

Although activation in an overall sense is kinetically similar, the underlying molecular processes in each fibre type may not be identical. Interestingly, the drug dantrolene sodium, which affects the S-D curve of frog twitch fibres¹⁴, possibly by interfering with Ca^{2+} release¹⁵, has no effect on the S-D relation for slow fibres (unpublished data).

If identical brief depolarisations lead to the same amount of Ca^{2+} released in both fibre types, one might expect, neglecting possible differences in thin filament Ca^{2+} sensitivities, similar numbers of perhaps both SR Ca^{2+} release sites as well as T-tubule:SR triad contacts in both types. Disposition of the internal membranes in slow fibres has been clarified with high voltage electron microscopy^{16–18}. Though preliminary, these results suggest that the number of triad (or dyad) 'feet' bridging the T:SR junctional gap in a given volume of muscle fibre may be quite similar for twitch and slow (C. H. Bailey and L. D. Peachey, personal communication). If the feet do subserve an important step in mechanical activation^{19,20}, similarities in activation kinetics described here may have an anatomical basis, and it may not be necessary to speculate on alternative pathways of activation in frog slow fibres.

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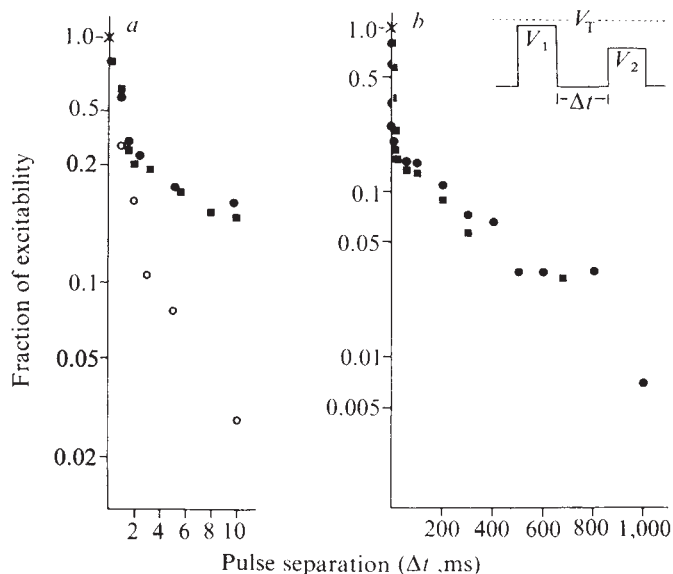


Fig. 2 Decay of mechanical excitability at 9 °C in twitch and slow fibres after a brief subthreshold pulse (V_1). Fractions of excitability remaining at a time Δt were estimated as: $[V_T - V_2(\Delta t)] / [V_T - V_2(0)]$, where $V_2(\Delta t)$ is membrane potential during the V_2 test pulse with pulse separation Δt , and $V_2(0)$ is membrane potential of V_2 with zero pulse separation. V_T is the usual threshold membrane potential for a single pulse of the same duration as V_2 . V_T was checked periodically during the experiment and did not change by more than a few mV. V_1 and V_2 pulses were of the same duration throughout: 5 ms in slow and 3 ms in twitch fibres. *a*, Fast excitability decay in one twitch (○) and two slow fibres (●, ■). Cross represents value shared by all fibres. Twitch fibre threshold returned to normal in about 20 ms. *b*, Slow excitability decay in the two slow fibres of (*a*). Threshold returned to the original V_T level only after 1000–2000 ms.

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Is thyroxine a regulatory signal for neurotubule assembly during brain development?

TUBULIN, the basic subunit of microtubules¹, may be assembled, *in vitro* directly from crude brain supernatants to microtubule-like structures^{2,3}. We have used this methodology to measure the initial rate of assembly *in vitro* in crude brain supernatants prepared from rats of different ages. Previous results^{4,5} provided clear evidence that at a late foetal stage or just after birth the concentration of tubulin in the brain is not the rate limiting factor for microtubule assembly. We assumed therefore that a factor other than tubulin is necessary to initiate the assembly process and that this factor is limiting in young brain. On the other hand, brain maturation is a slow post-natal process in the rat and it is generally believed that this process is under thyroid hormone control. We report here that microtubule formation during brain development requires the presence of an initiation factor and that the onset of this factor is under thyroid hormone control.

Thyroid hormones have a profound effect on brain development; for example, congenital hypothyroidism produces severe mental retardation (cretinism) in man and animals. Treatment with thyroid hormones after birth accelerates the development of innately organised behaviour and of electrocortical activity⁶. Histological alterations observed during hypothyroidism^{7–9} mainly concern the development of cellular processes and dendrites. These effects are associated with biochemical changes; the protein content and protein synthetic activity of the brain are reduced^{10,11}. None of these observations, however, is directly related to a possibly more specific effect of thyroid hormones during brain development—the increase in the number of connections, for instance of dendrites spines of cortical neurones and of Purkinje cells in the cerebellum⁷.

Microtubules are good markers of brain connections as they are very abundant in these structures^{12–14}. At early stages of development, however, tubulin polymerises very poorly *in vitro* in conditions described by Weisenberg² and Shelanski³. Figure 1 shows, for instance, that in 3-d-old rat brain preparations the rate of *in vitro* microtubule assembly is markedly lower than in those of 15-d-old rats, and also that the lag period is increased;

the critical concentration¹⁵ of tubulin at which microtubule assembly can be observed *in vitro* was also increased (not shown). This difference in rates depends on the fact that, at early stages of development, a factor which is needed to initiate the assembly process, is limiting^{16,17}. This factor has been tentatively identified¹⁶ with the protein described by Weingarten *et al.*¹⁸. Another possibility would have been that the young brain supernatants contain an inhibitor of the microtubule assembly; this possibility was eliminated, as reported before⁵, by mixing supernatants from 30-d-old and newly born rats: there was no inhibition of assembly in these conditions. In addition, mixing supernatants of newly born rats with very small amounts of sonicated fragments of adult preparations resulted in a normal rate of assembly⁵.

Severe hypothyroidism can be produced in the rat by treatment with antithyroid drugs during pregnancy and after birth⁷. Pregnant females were force-fed from the 18th d of gestation until 15 d after delivery with 3-methylthiouracil (MTU); thus MTU was supplied to the foetus during pregnancy and to the newborn rats through the milk of the mother during lactation. At 15 d after birth the newborn rats were clearly hypothyroid—the body weight was 28.8 ± 2.18 g (control rats 34.16 ± 0.3 g). The brain weight was also reduced but only by 20%. The tubulin contents of these supernatants evaluated by a modification⁴ of the ³H-colchicine binding method¹⁹ were very similar (controls, 2.87 mg; MTU-treated 2.74 mg tubulin per g wet weight).

In vitro microtubule assembly in brain supernatants was followed using the turbidimetric method of Shelanski *et al.*³ Figure 1 shows that, after MTU treatment, microtubules polymerised very poorly *in vitro* when the same tubulin concentration was used in the assays (15-d-old MTU treated rat brain supernatants) and in the controls (15-d-old normal rat brain supernatants). The initial rate of assembly markedly decreased and the latency increased. The kinetics of assembly resembled those observed 3 d after birth, suggesting that hypothyroidism produces a retardation or a blockage of the ability of tubulin to assemble to microtubules. These results suggest that thyroid hormones have a greater effect on the

Fig. 1 Turbidimetry time course of supernatants from 3 and 15-d-old euthyroid rats and 15-d-old hypothyroid rats. The supernatants were obtained after homogenisation of rat brains in a reassembly buffer containing: 0.1 M morpholino-ethane sulphonate (pH 6.5, 1 mM GTP, 0.5 mM MgCl₂, 1 mM EGTA, 0.1 mM EDTA, 1 mM β-MSH at 4 °C and centrifugation at 105,000g for 1 h at 4 °C. Glycerol (final concentration 4 M) was added after centrifugation. Turbidimetry (*A* at 345 nm) time course at 37 °C was followed with supernatants dilutions containing the same (0.940–1.040 mg ml⁻¹) amounts of tubulin, measured as before¹³. 15-d-old hypothyroid rats were obtained by a treatment of the mothers with MTU from the 18th d of gestation (50 mg per day given by force feeding²) until the day of killing. ★, 3-d-old euthyroid rat brain supernatant (tubulin 0.94 mg ml⁻¹); ○, 15-d-old euthyroid rat brain supernatant (control) (tubulin 1.05 mg ml⁻¹); ●, 15-d-old hypothyroid rat brain supernatant (tubulin 0.95 mg ml⁻¹).

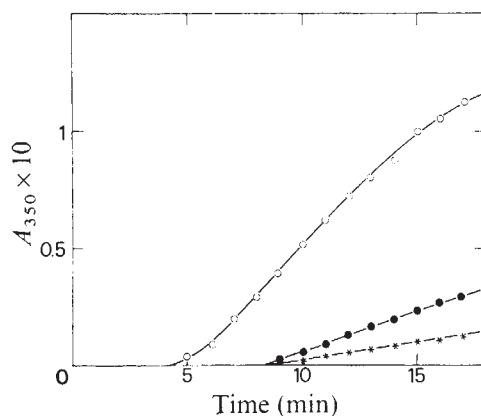
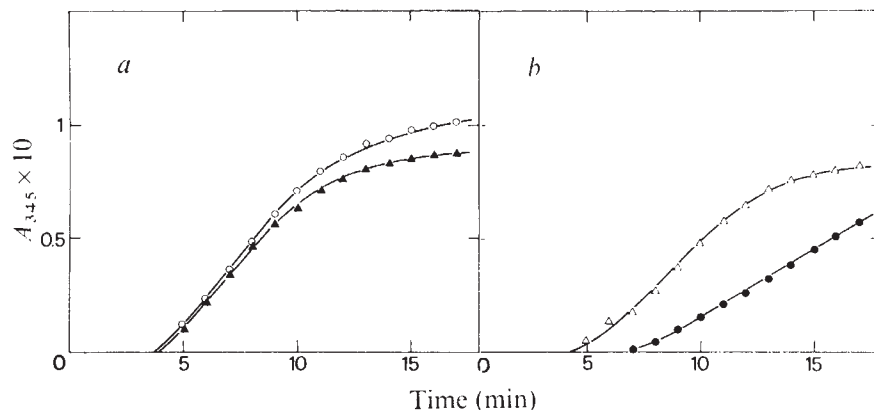


Fig. 2 Effect of thyroxine treatment on the turbidimetry time course of 15-d-old euthyroid and hypothyroid rat brain supernatants (obtained as described in Fig. 1). *a*, $\bigcirc$, 15-d-old euthyroid rat brain supernatant (tubulin 1.04 mg ml^{-1}); $\blacktriangle$, thyroxine-treated 15-d-old rat brain supernatant (tubulin 0.95 mg ml^{-1}). *b*, $\bullet$, 15-d-old hypothyroid rat brain supernatants (MTU treated (tubulin 0.95 mg ml^{-1})); $\triangle$, thyroxine-treated 15-d-old hypothyroid rat brain supernatants (tubulin 1.04 mg ml^{-1}).



initiation process of microtubule assembly than on the tubulin content of the brain.

To test whether the observed low polymerisation was due to the lack of thyroid hormones or to a toxic effect of MTU, normal rats and animals rendered hypothyroid by MTU

treatment were injected subcutaneously just after birth with $2 \mu\text{g}$ DL-thyroxine per day during 6 d and with $4 \mu\text{g}$ thyroxine per day during the last 9 d before killing. For the hypothyroid rats, thyroxine treatment produced a small increase in the tubulin concentration (MTU + thyroxine $4.04 \text{ mg tubulin per g wet weight}$). Figure 2 shows typical experiments where the effect of thyroxine treatment on the rate of *in vitro* microtubule assembly was studied with identical concentrations of tubulin in the assays and in the controls. Thyroxine treatment had no effect on the control (Fig. 2a), whereas the initial rate of assembly was markedly increased and the latency markedly reduced for the MTU-treated rat brain preparations (Fig. 2b).

The effects of the addition of the τ factor, an initiator of microtubule assembly¹⁸ were also studied. The τ factor was added in increasing amounts to 40- and 15-d-old brain supernatants prepared from euthyroid and hypothyroid rats (Fig. 3). The addition of the τ fraction to the adult supernatant did not produce an increase on the rate of assembly (Fig. 3a), suggesting that the adult brain supernatant contains saturating amounts of τ factor. In contrast, addition of τ factor to the 15-d-old brain supernatants produced an increase in rate of assembly (Fig. 3b). Thus 15 d after birth the τ factor concentration is still limiting. The effect of the τ fraction on the brain supernatants from MTU-treated rats was even greater; the initial rate of assembly increased to the level of the controls and the lag period decreased (Fig. 3c). Thus hypothyroidism probably produces a decrease in the concentration of the τ factor and not in the amount of tubulin. We reported before¹⁶ that addition of a τ preparation significantly increased the rate of microtubule assembly of newly born (3-d-old) rat brain supernatants.

These observations suggest that microtubule formation during normal brain development requires the presence of thyroid hormones. If, as reported by others^{20,21}, microtubule assembly is required for axon growth and for the formation of nerve connections, the results of this work might contribute to the understanding of the effects of thyroid hormones in brain development and in the aetiology of cretinism.

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Fig. 3 Effect of τ factor on the turbidimetry (345 nm) time course at 37°C of adult euthyroid, 15-d-old euthyroid and hypothyroid rat brain supernatants. Experimental conditions were as described in Fig. 1. τ factor was purified as before¹⁷. *a*, Adult rat brain supernatant (tubulin 0.88 mg ml^{-1}) in the absence ($\triangle$) and in the presence ($\star$) of τ factor (0.23 mg ml^{-1}). *b*, 15-d-old euthyroid rat brain supernatants (tubulin 0.78 mg ml^{-1}) in the absence ($\bigcirc$) and in the presence of τ factor ($\blacktriangle$, 0.115 mg ml^{-1}), ($\star$, 0.23 mg ml^{-1}). *c*, 15-d-old hypothyroid rat brain supernatants (tubulin 0.73 mg ml^{-1}) in the absence ($\bullet$) and in the presence of τ factor ($\blacktriangle$, 0.115 mg ml^{-1}), ($\star$, 0.23 mg ml^{-1}).

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Evidence from oxygen exchange measurements for a cooperative interaction between the two heads of myosin

THE present view of the molecular mechanism of muscle contraction¹ does not explain why myosin has two structurally similar globular heads². Each of these heads can interact with actin and with ATP, and each seems to hydrolyse ATP at about the same rate³. To investigate possible functional differences between the two heads of myosin, we have compared the rates of intermediate oxygen exchange⁴⁻⁶ catalysed by heavy meromyosin (HMM), which has two heads, and subfragment 1 (S1), which has one head, at different levels of actin activation. This oxygen exchange occurs between water and the γ -phosphoryl groups of two enzyme-nucleoside phosphate intermediates along the pathway of coupled ATP hydrolysis⁷. Thus, measurements of oxygen exchange can be used to probe the kinetics of intermediate steps in the hydrolytic and contractile mechanism.

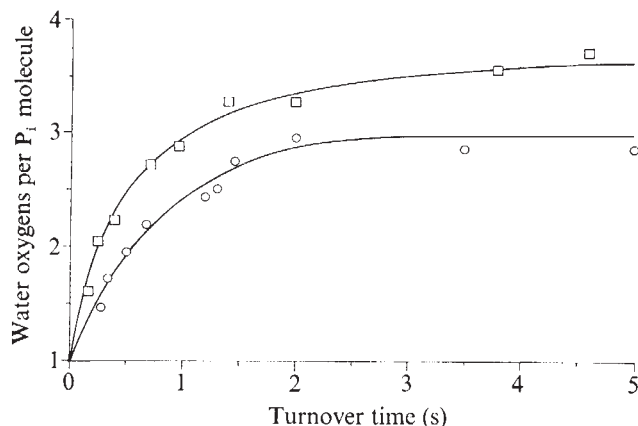


Fig. 1 Incorporation of ^{18}O from water into P_i as a function of turnover time. The assay mixture contained 25 mM KCl, 25 mM Tris buffer (pH 7.4), 5 mM MgCl_2 , 5 mM ATP and appropriate concentrations of proteins in ^{18}O water at 23 °C. S1 was prepared from rabbit myosin by the method of Margossian *et al.*¹² with Mg^{2+} present instead of EDTA to preserve the DTNB light chain. HMM was prepared by the method of Holt and Lowey¹³. Actin was prepared from acetone-dried muscle powder by the method of Spudich *et al.*¹⁴. P_i was isolated as KH_2PO_4 essentially by the method of Dempsey *et al.*¹⁵. The ^{18}O content of P_i was determined by the method of Boyer and Bryan¹⁶. At each concentration of actin, the rate of hydrolysis was obtained from the slope of a linear rate curve and converted to a turnover number (molecules of ATP hydrolysed per second per active site). The turnover time on the abscissa is the reciprocal of this number. Actin markedly activated both fragments up to more than 100-fold at the highest levels. In the conditions of these experiments, with either fragment, the turnover rate increased linearly with the actin level; falling below the line only slightly at the highest level. To obtain a given average turnover time per head, about the same concentration of actin was needed with HMM as with S1. Turnover times were calculated using the equivalent weights of 120,000 per head for S1 ($\square$) and 170,000 per head for HMM ($\circ$).

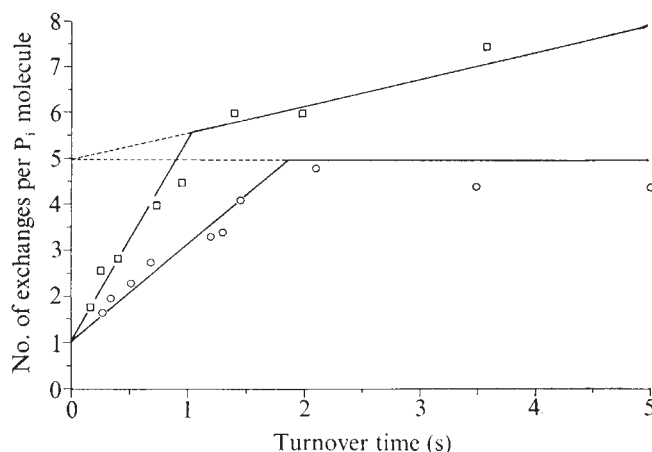


Fig. 2 Calculated number of exchange reactions as a function of turnover time. Conditions are given in Fig. 1. The number of exchange reactions was calculated using the equation of Wolcott and Boyer⁸

$$\frac{\text{water oxygens}}{\text{P}_i \text{ molecule}} = \sum_{n=1}^n (3/4)^{n-1}$$

where n is the number of exchange reactions. In each curve the initial slope, which extrapolates back to one exchange (the one added by the first cleavage of bound ATP) gives the average rate of exchange for the three exchangeable oxygens per P_i molecule. The upper shallow slope estimates the average rate of exchange after two oxygens per P_i molecule have been labelled, that is, when their exchange no longer has any significant or measurable effect on ^{18}O incorporation. Thus, the initial slope minus the final slope is the rate of exchange for one that exchanges slowly. $\circ$, HMM; $\square$, S1.

Any difference in the rate of oxygen exchange indicates either a difference in the lifetimes of the exchanging intermediates, or in the kinetic constants of the exchange reaction itself^{4-6,8}.

The results reported here show that HMM catalysed approximately half as much oxygen exchange per second per head as did S1. This indicates that in HMM there may be some cooperative interaction between the heads which effectively inhibits some part of the exchange mechanism. Since oxygen exchange is often correlated with the actin-activated ATP hydrolysis coupled to contraction^{4-6,9} such an effect could reflect a cooperative action of the two heads in the generation of force or the regulation of contraction.

Actin decreases the turnover time of ATP hydrolysis by myosin and its fragments. It does this by activating the rate-limiting step in the hydrolytic pathway. This rate-limiting step follows oxygen exchange^{1,7}. Therefore, the greater the actin concentration, the shorter the turnover time, and the shorter the lifetime of the exchanging intermediates. Figure 1 shows how the incorporation of ^{18}O from water into P_i changed when the turnover time was changed by the concentration of actin in the reaction solution. Figure 2 shows the calculated number of exchange reactions as a function of turnover time. The method for calculating the number of the reactions from the measured incorporation is given in Fig. 2. The slope of each curve in Fig. 2 gives an estimate for the average rate of oxygen exchange, that is, the number of exchanges per second of turnover time.

The use of such plots to study the kinetics of oxygen exchange is described in our recent paper¹⁰. From the earlier experiments with S1 (ref. 10) we concluded that the four oxygens in each γ -phosphoryl group do not all exchange at the same rate. To summarise our interpretation: the first oxygen is incorporated at virtually zero time by the very fast initial cleavage of the γ -phosphoryl group of bound

ATP (ref. 11); two oxygens exchange at a relatively fast rate by the mechanism of Bagshaw and Trentham¹¹, that is, by a cycle that first reverses the cleavage of the nucleotide and then recleaves it; each such cycle can cause the exchange of an oxygen atom, if the oxygens can rotate in space so that the one added by cleavage may be different from the one lost by reverse cleavage; the fourth oxygen exchanges very slowly, and this slow exchange indicates that one oxygen may be severely restricted from entering into the exchange cycle by its binding to the protein.

By comparing the slopes in Figs 1 and 2 it became evident to us that HMM, compared with S1, catalysed oxygen exchange at about half the rate per head. In general there are several alternatives for interpreting this finding but one that may have considerable significance to muscle contraction is the clear possibility that only one head of HMM at a time is in a conformation that can catalyse oxygen exchange. This would effectively reduce by half the measured rate of exchange per head. Such a cooperative effect that holds each head in a somewhat different conformation, that is, it does not allow them to take the same conformation at the same time, may coordinate the working of the two heads in the contraction and relaxation of muscle. Work is continuing with other fragments and other forms of S1 to test further whether the marked difference between the oxygen exchange rates of HMM and S1 heads reported here reflects a functionally important property of myosin.

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Haemin protects Met-tRNA_f binding activity of isolated reticulocyte ribosomes from inactivation by protein kinase

RETICULOCYTE lysates incubated in the absence of haemin stop synthesising proteins after a few minutes due to the accumulation of a translational inhibitor (TI) which blocks initiation of protein synthesis¹⁻³. This block is characterised by a depletion of initiator tRNA (Met-tRNA_f) bound to 40S ribosomal subunits^{4,5}. It has been reported that protein kinase activity is associated with TI which has been partially purified from reticulocyte lysates⁶. Addition of the initiation factor eIF-2 (previously designated IF-E2 or IF-MP), which binds Met-tRNA_f and GTP, can overcome the block in initiation which develops in reticulocyte lysates incubated in the absence of haemin or with added TI^{7,8}. Phosphorylation of eIF-2 by a protein kinase present in TI preparations has been demonstrated (refs 9-12 and P. Farrel, K. Balkow, R. Jackson and T. Hunt, personal

communication), and it has been proposed that phosphorylated eIF-2 is inactive^{9,9}. We have investigated the mechanism by which haemin stabilises initiation of protein synthesis by examining the ability of TI to inhibit binding of Met-tRNA_f to ribosomes obtained from reticulocyte lysates supplemented with haemin or from unsupplemented lysates, and the corresponding pattern of ribosome-associated proteins phosphorylated by the protein kinase(s) present in the TI preparation. We have established a correlation between the phosphorylation of proteins associated with native 40S ribosomal subunits and the loss of binding activity of the ribosomes.

TI was prepared from unsupplemented reticulocyte lysates, as described before⁶. Protein synthesis by reticulocyte lysates supplemented with 50 μ M haemin is inhibited 80% by 25 μ g of TI preparation in a 50- μ l assay (unpublished work of Sarma, M. and Baglioni, C.). Ribosomes isolated from reticulocyte lysates (Fig. 1) were incubated with (³H)Met-tRNA_f and GTP. The amount of initiator tRNA bound was measured by passing the incubation mixture through nitrocellulose filters directly or by fractionating the incubation mixture on gradients¹³. Gradient fractions were passed through filters, and Met-tRNA_f was found bound to ribosomes only (data not shown). This binding is GTP dependent and eIF-2 is the only initiation factor reported to bind Met-tRNA_f in a GTP-dependent fashion¹⁴.

Inhibition of Met-tRNA_f binding by TI was assayed by

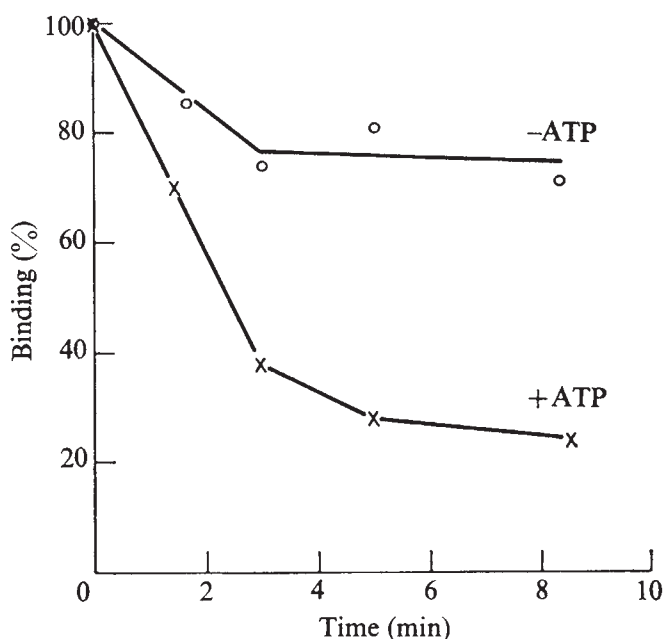


Fig. 1 Time course of inhibition of (³H)Met-tRNA_f binding to ribosomes by a translational inhibitor (TI) in the presence or absence of ATP. Reticulocytes were obtained from rabbits made anaemic with 1-acetyl-2-phenylhydrazine. TI was prepared from the postribosomal supernatant of unincubated reticulocyte lysates, following the procedure of Levin *et al.*⁶. The fraction eluted from DEAE-cellulose at 0.24 M KCl was used. Ribosomes were prepared as previously described¹⁴, except that all solutions contained 1 mM dithiothreitol. Stripped Met-tRNA_f from rabbit liver (Grand Island Biological Co.) was charged with (³H) methionine (3.7 Ci mmol⁻¹) using an *E. coli* aminoacyl synthetase (Miles). Approximately 40 μ g of ribosomes were incubated in 35 μ l incubation mixtures containing buffer A (110 mM KCl, 20 mM Tris-HCl pH 7.5, 5 mM MgCl₂, 1.2 mM dithiothreitol), 12 μ g of TI and 40 μ M ATP (×) or no ATP (○). After incubation at 30 °C for the time indicated on the abscissa 3 pmol of (³H)Met-tRNA_f (7,500 c.p.m.) and 1 mM GTP in 15 μ l of buffer A were added and the incubation continued for 5 min at 24 °C. The reactions were stopped and the (³H)Met-tRNA_f bound determined by the nitrocellulose filter assay previously described¹⁴. Samples which were not incubated at 30 °C before the addition of Met-tRNA_f bind about 0.3 pmol of Met-tRNA_f. Binding is expressed as the percentage of binding activity relative to such samples.

pre-incubating ribosomes with TI for various times, then adding Met-tRNA_i and GTP (Fig. 1). Only some loss of binding activity is observed on incubation of ribosomes with ATP or TI (Table 1). Addition of 40 μ M ATP together with 12 μ g of TI however, results in 75% inhibition of the binding of Met-tRNA_i (Fig. 1). We have, therefore, shown an ATP requirement for the inhibition, suggesting that the protein kinase activity of TI is directly responsible for the inhibition of Met-tRNA_i binding to ribosomes in the assay described. The ATP requirement can be shown because ribosomes have been isolated through a sucrose cushion, thus removing endogenous ATP. Incomplete removal of ATP may be responsible for the slight loss of Met-tRNA_i binding activity observed in incubations with no added ATP (Table 1). Addition of 1.5 μ M cAMP has no effect on the loss of Met-tRNA_i binding activity (data not shown), indicating that the inhibitor protein kinase is not stimulated by cyclic AMP.

Inhibition of Met-tRNA_i binding by TI and 40 μ M ATP is incomplete, leaving about 25% of the original activity of control ribosomes (Fig. 1). This residual binding may be due to eIF-2 activity which is resistant to inactivation by TI. A higher resistance to inactivation by TI is indeed observed with ribosomes prepared from lysates supplemented with haemin (Fig. 2). Loss of Met-tRNA_i binding activity is consistently less for these ribosomes than for ribosomes prepared from unsupplemented lysates (Table 1).

Since inactivation of Met-tRNA_i binding in the presence of TI is ATP-dependent and protein kinase activity is present in TI preparations, it is reasonable to propose that specific initiation factor(s) involved in the binding of initiator tRNA to ribosomes are inactivated by phosphorylation. We have, therefore, used (³²P- γ)ATP to phosphorylate reticulocyte ribosomal proteins and have examined the proteins by electrophoresis on polyacrylamide gels. Figure 3 shows the autoradiographic pattern obtained when ribosomes are incubated with TI in conditions which lead to the inhibition of Met-tRNA_i binding (track 1). Many of the bands correspond to polypeptides present in the TI preparation (track 5). Notable among the phosphorylated polypeptides of ribosomes (track 1) are those of MW 67,000, 53,000 and 38,000. The 67,000 polypeptide has a MW similar to that of initiation factor IF-M1, which has been reported to be 65,000 (ref. 15). The other bands may correspond to two of the three polypeptides comprising eIF-2.

Table 1 ATP dependence of the inhibition of Met-tRNA_i binding by a translational inhibitor (TI) and protection from inactivation by haemin

Exp. no.	+ ATP	+ TI	Inhibition (%)	
			+ TI + ATP	+ TI + ATP + haemin
1	21	19	71	43
2	30	—	73	51
3	24	—	78	—
4	—	11	54	—
5	—	9	64	—
6	—	—	61	50

Ribosomes were incubated 5 min at 30 °C with 40 μ M ATP, where indicated, and/or 12 μ g TI, and then with (³H)Met-tRNA_i and GTP as indicated in Fig. 1. Determination of the bound Met-tRNA_i was as described in the legend of Fig. 1. Ribosomes from the incubations designated as '+ haemin' were obtained from reticulocyte lysates supplemented with 50 μ M haemin; all other incubations contained ribosomes from unsupplemented lysates. Inhibition is expressed as the percentage of binding activity lost relative to samples not incubated at 30 °C before addition of Met-tRNA_i and GTP. Different preparations of ribosomes obtained from different lysates were used for each experiment. Addition of TI and ATP after incubation of ribosomes with Met-tRNA_i and GTP does not decrease the amount of bound Met-tRNA_i. Protein synthesis in cell lysates is 50% inhibited by 10 μ g of TI in a 50- μ l assay¹³, and Met-tRNA_i binding is inhibited 50% by 5 μ g of TI in the assay described in the legend of Fig. 1.

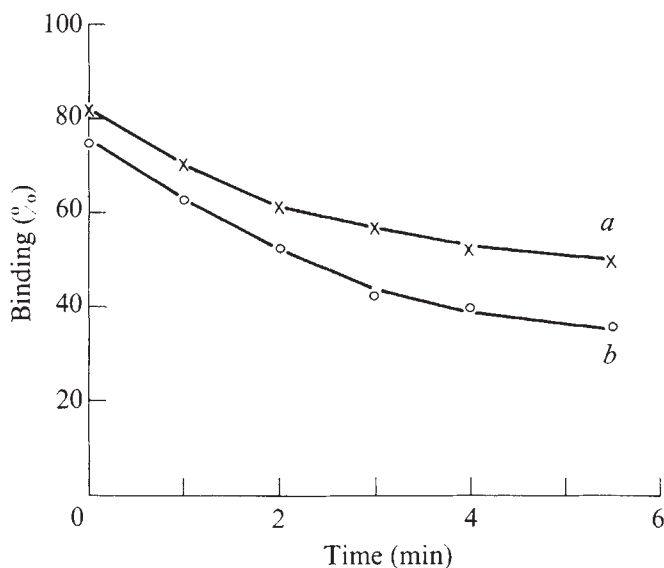


Fig. 2 Time course of haemin protection of (³H)Met-tRNA_i binding to ribosomes from inactivation by TI. Approximately 40 μ g of reticulocyte ribosomes from either lysates supplemented with 50 μ M haemin (a) or unsupplemented lysates (b) were incubated with 40 μ M ATP and TI at 30 °C for the time indicated on the abscissa as described in Fig. 1. Then (³H)Met-tRNA_i and GTP were added, the incubation continued at 24 °C for 5 min, and the samples processed as described in Fig. 1. Binding is expressed as a percentage of the amount of (³H)Met-tRNA_i bound relative to samples incubated without TI.

Phosphorylation *in vitro* of each of the three subunits of eIF-2 by protein kinases has been reported (refs 9–12, and P. Farrel *et al.*, personal communication). Moreover, phosphorylation of the 38,000 subunit may be responsible for inhibition of initiation of protein synthesis (ref. 11, and P. Farrel *et al.*, personal communication). Phosphorylation of other initiation factors which stabilise the association of Met-tRNA_i with 40S ribosomal subunits¹⁶ may also play a role in the inhibition of Met-tRNA_i binding.

A difference in the pattern of phosphorylated polypeptides is observed between ribosomes prepared from lysates supplemented with haemin (track 2) and those from unsupplemented lysates (track 1). A polypeptide of MW 53,000 is phosphorylated to a much greater extent in the ribosomes from unsupplemented lysates. This polypeptide is associated with native 40S ribosomal subunits. Incubation of these subunits with TI and (³²P- γ)ATP results in the phosphorylation of several polypeptides, among which is the 53,000 polypeptide. Addition of 50 μ M haemin to the incubation medium markedly decreases the phosphorylation of this polypeptide (Fig. 3, tracks 3 and 4). The 53,000 MW polypeptide is not associated with salt-washed 40S subunits derived from polysomes (unpublished work of Sarma, M. and Baglioni, C.). Since most eukaryotic initiation factors are associated with native 40S ribosomal subunits^{17,18}, it is likely that the polypeptides phosphorylated by TI which are associated with these subunits, but not with salt-washed 40S ribosomal subunits, are initiation factors.

The data presented here show that a certain amount of the Met-tRNA_i binding capacity of ribosomes is resistant to inactivation by TI. Ribosomes from haemin-supplemented lysates show a greater resistance to inactivation by TI than those from unsupplemented lysates. This increased resistance due to haemin is correlated with a decrease in phosphorylation of a specific polypeptide of MW 53,000.

We have established that haemin co-sediments with ribosomes prepared from cell extracts supplemented with 50 μ M haemin. By analysing the visible absorption spectrum of these ribosomes, we find the typical spectrum of a haemin-protein complex with a maximum absorption at 390 nm.

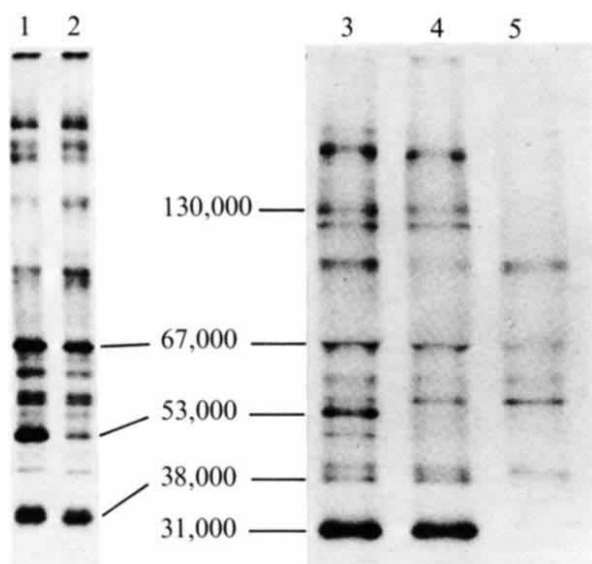


Fig. 3 Polyacrylamide gel electrophoresis of (^{32}P)-labelled ribosomal proteins. For tracks 1 and 2, 40 μg of ribosomes from either lysates supplemented with 50 μM haemin (2) or unsupplemented lysates (1) were incubated with TI as described in Fig. 1, except that 40 μM (^{32}P - γ)ATP (15 Ci mmol $^{-1}$) and 1.5 μM cyclic AMP but no (^3H)Met-tRNA $_f$ or GTP were added. Omission of cyclic AMP results in no change in the pattern of phosphorylated proteins at this ATP concentration. For tracks 3, 4 and 5, 25 μg of TI were incubated with 30 μg of BSA, 400 μM ATP, 5 μCi of (^{32}P - γ)ATP, 1.5 μM cyclic AMP, 25 mM HEPES-KOH (pH 7.2), 6 mM Mg(OAc) $_2$, 20 mM KCl, and the following additions: 3, 16 μg of native 40S ribosomal subunits prepared according to Freinstein and Blobel 18 ; 4, 16 μg of native 40S subunits and 50 μM haemin; 5, no additions. Incubations were at 30 $^{\circ}\text{C}$ for 30 min in a 50- μl volume. Omission of cyclic AMP resulted in a significant decrease in the phosphorylation of only two bands, one of MW 31,000 which is a ribosomal protein present in salt-washed 40S ribosomal subunits 12 and the other of approximate MW 130,000. Reactions were stopped by adding an equal volume of 2 $\times$ electrophoresis buffer. Samples were fractionated by electrophoresis on 8-cm gel slabs, made with 12.5% acrylamide and 0.1% bis-acrylamide at pH 8.7, with a 2-cm, 5% acrylamide, 0.13% bis-acrylamide stacking gel at pH 6.8 according to Laemmli 22 with bis-acrylamide concentrations from Blattler *et al.* 23 . Electrophoresis was at room temperature for 1 h at 50 V, then for 3.5 h at 150 V. Gels were stained with Coomassie Brilliant Blue, dried, and autoradiographed for 1–3 days using Kodak RP Royal X-Omat film. The MW included have been calculated by running in separate tracks marker polypeptides of known MW.

Ribosomes obtained from cell extracts unsupplemented with haemin do not show such an absorption at 390 nm. Haemin may (i) render the 53,000 MW polypeptide a less accessible substrate for a protein kinase, (ii) increase the activity of a phosphatase specific for this polypeptide, or (iii) inhibit a kinase which specifically phosphorylates this polypeptide. Haemin may modulate the activity of a protein kinase and of a phosphatase, as suggested by Traugh 19 . The finding that haemin promotes the aggregation of inactive low MW initiation factors into active high MW complexes 20 , suggests, however, that haemin may directly change the conformation of initiation factors and possibly render them more resistant to inactivation by protein kinases.

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Does 5S RNA function by a switch between two secondary structures?

THE structure of 5S RNA has been a lively field for experiment and speculation, with no definitive resolution of the problem in sight (see refs 1 and 2 for reviews). Fox and Woese 3 suggested comparative sequence analysis of prokaryotic 5S RNAs as a method for locating base paired regions, an appealing procedure because of its similarity to the method used to substantiate the cloverleaf model for tRNA. Its logic states that if all known 5S RNA sequences contain complementary bases at a site, a double helix probably exists there in the functional form. We propose that comparative sequence analysis suggests two different functional structures for prokaryotic 5S RNA. One of them has been introduced by Fox and Woese 3 , and its secondary structure is based on four conserved helices: the 'molecular stalk' (*Escherichia coli* residues 1–10 and 119–110), the weak 'tuned helix' (18–23/65–60), the 'common arm base' (31–34/51–48) and the 'prokaryotic loop' (82–86/94–90). The base paired structure that results is consistent with enzymatic digestion 14 and chemical modification 1,5 results on native 5S RNA, if allowance is made for protection of residues by tertiary bonding as well as secondary structure. This 'model', however, does not explain the apparent conservation of a double helix (33–39/88–82 in *E. coli*) (Table 1) which is sterically inconsistent with two of the helices listed (the common arm base and the prokaryotic loop). Fox and Woese 3 noted this conservation but considered it unimportant because of the variable helix length and the conflict with the other two helices.

Our proposal begins with the observation that the alternate conserved helix explains the enzymatic degradation pattern 4 of the 'denatured' or B form of 5S RNA (ref. 13), whereas the Fox and Woese model does not. Hence the alternate conserved helix, which we term the 'central helix', may be part of the secondary structure of the B form. Since the central helix is conserved over all known sequences, we conclude that it is functionally significant. Therefore the finding of two conformational forms of 5S RNA is neither accident nor artefact, but an indication of an important conformational balance which allows rearrangement of 5S RNA secondary structure during protein biosynthesis. The fact that only the A form has yet been isolated from native ribosomes may be because of instability of the B form except in the elongation factor G/GTP/ribosome complex.

Figure 1 shows the two models. The crucial observations that distinguish them derive from the nuclease digestion experiments of Jordan 4 . He found a fragment of the B form containing residues 25–41 and 96–80, clearly indicating that those

Table 1 The neighbourhood of the central helix in prokaryotic 5S RNA (frames)

E.coli ⁸	... ²⁵ UCCCACCU ³⁰	GACCCCA	⁴⁰ UGC CGAA		⁷⁵ GGU ⁸⁰ AGUG	UGGGGUC	⁹⁰ UCCCCAUG...
Photobacterium 8265 ³	... ³⁰ ACCCACCU	GAUCCC	⁴⁰ UGC CGAA		⁸⁰ CGU ⁸⁰ AGUGU	GGGGUU	⁹⁰ UCCCCAUG...
Pseudomonas fluorescens ⁷	... ³⁰ AA CACCU	GAUCCCA	⁴⁰ UCC CGAA		⁸⁰ GGU ⁸⁰ AGUG	UGGGGUU	⁹⁰ UCCCCAUG...
Clostridium pasteurianum ⁹	... ²³ UAACACUC ³⁰	CUUCCC	⁴⁰ AUUC CGAA		⁷³ GGUACUGCAG ⁸⁰	GGGAAG	⁹⁰ CCUGUG...
Bacillus megaterium ⁹	... ³⁰ UCACACCC	GUUCC	⁴⁰ CAUAC CGAA		⁷³ GGU ⁸⁰ AGUU	GGGAC	⁹⁰ UUUGUCCUG...
B. stearothermophilus ¹⁰	... ³⁰ AAACACCC	GUUCC	⁴⁰ CAUCC CGAA		⁷³ GGU ⁸⁰ AGUU	GGGGC	⁹⁰ CAGCGCCCCUG...
B. subtilis ¹⁰	... ³⁰ UCACACCC	GUUCCC	⁴⁰ AUAC CGAA		⁷³ GGU ⁸⁰ AGUC	GGGGGU	⁹⁰ CUUCCCCUG...
B. licheniformis ¹¹	... ³⁰ UCACACCC	GUUCUCA	⁴⁰ UGC CGAA		⁷³ GGU ⁸⁰ AGU	UGGGGGC	⁹⁰ UUCCCCUG...
A. nidulans ¹²	... ²⁴ AACCACUCUG ³⁰	ACCC	⁴⁰ CAUCC CGAA		⁷⁵ GAUAGCUCCC ⁸⁰	GGGU	⁹⁰ AGCCGAUCG...

sections are bonded together. This feature is found in our B-form model but not in the A-form model. The only double helices included in Fig. 1 are those which can be justified by comparative sequence analysis. The 'tuned helix', though sterically feasible in the B form, is not included because chemical modification results⁵ indicate that G₆₁ is accessible for modification in the B form. The high activation energy necessary for the conversion between the two forms¹⁴ corresponds well to the melting of the specific helices.

We feel that most of the remaining structural organisation of both 5S RNA forms is probably not provided by Watson-Crick base pairing, but rather by tertiary interactions of an unknown nature. There is, however, no definitive method for distinguishing secondary and tertiary structures in RNA molecules. NMR measurement of protons in base pairs is a promising technique, but is complicated by the contribution of resonances from protons in tertiary bonds with chemical shifts similar to protons in standard base pairs¹⁵⁻¹⁷. Hence we can discount detailed models of 5S RNA structure based on NMR measurements which ignore possible contributions from tertiary bonds¹⁸. Our studies of conformational equilibria and dynamics in 5S RNA (in preparation) provide circumstantial evidence favouring extensive tertiary structure in 5S RNA.

The detailed mechanism of the 5S RNA conformational switch, and the role of GTP hydrolysis and ribosomal proteins^{19,20}, are subjects for potentially endless speculation. We limit ourselves to one observation. Figure 1 shows the highly conserved region G₇₂-G₈₃, which has recently been found to be complementary to a 23S RNA sequence²¹; also nucleotides CGAA in position 43-46 have been proposed^{22,23} as a tRNA binding site on the basis of their complementarity with the tRNA sequence T^ψCG. Note that the first base of the central helix precedes this CGAA sequence by exactly 10 nucleotides in all species but the blue-green alga *Aspergillus nidulans*, where both helix and distance are shorter by one nucleotide. The A ⇌ B conformational switch, as represented in Fig. 1, places the tRNA binding site adjacent to the 23S RNA in the B form, but free to move away from that position in the A form. A switch from one conformation to another, possibly induced by the binding and release of the elongation factor G, could cause movement of the tRNA relative to the 23S RNA. As the migration of the mRNA in the ribosome is probably induced by the tRNA (ref. 24), a process of this sort is likely to be part of the translocation step in protein biosynthesis.

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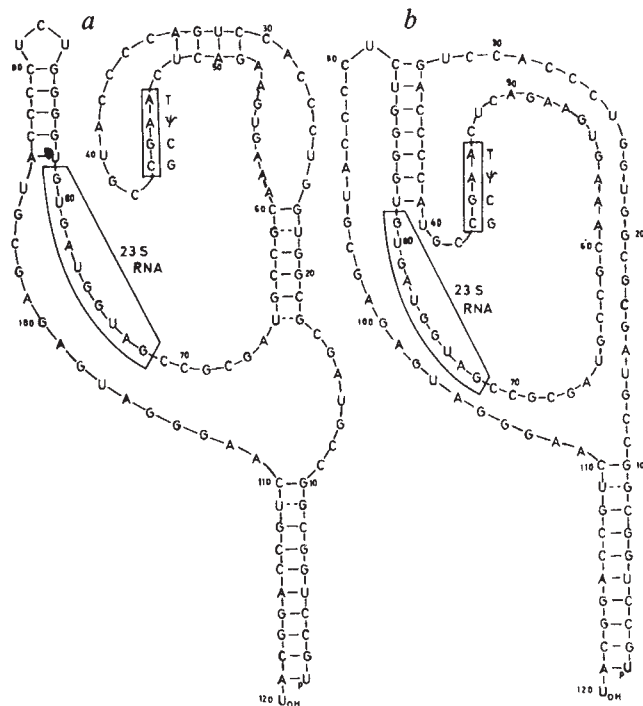
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Fig. 1 a, A-form and b, B-form conformations of prokaryotic 5S RNA. Sequences complementing 23S RNA and tRNA segments are indicated.



matters arising

Assortative mating

VETTA¹ claims that Fisher's² well known formulae for the correlations between relatives under assortative mating are incorrect, and he presents alternative formulae for the parent-child and sib-sib correlations. I intend to publish elsewhere a simplified proof of Fisher's results, but it seems worthwhile to indicate here why I am not convinced by Vetta's argument.

Consider for simplicity the case in which there is no dominance and no environmental variability, so that all the variance is additive genetic. The alternative formulae for the parent-child correlation, which is in this case the same as the sib-sib correlation, are as follows

$$\text{Fisher: } \frac{1}{2}(1+\mu), \text{ and} \\ \text{Vetta: } \frac{1}{2}[1+\mu(1-\mu)^2]$$

where μ is the correlation between husband and wife. There is nothing in Fisher's model which restricts attention to positive, as opposed to negative, assortative mating, so that μ may meaningfully take any value between -1 and $+1$. Fisher's correlation increases linearly from 0 to 1 as μ increases from -1 to $+1$, which seems reasonable. Vetta's correlation is negative when $\mu < -0.47$, which seems implausible, and is less than -1 when $\mu < -0.87$, which is impossible. Even if we confine our attention to positive values of μ the behaviour of Vetta's correlation is strange, since it increases from 0.5 to 0.574 as μ increases from 0 to $\frac{1}{2}$, and then decreases back to 0.5 as μ increases further from $\frac{1}{2}$ to 1; I find it difficult to understand how the correlation can fail to be an increasing function of μ .

It is also quite easy to prove, at least to my satisfaction, that Fisher's correlation is correct in this case. Elementary genetic considerations show that the expected value for the child given full information about the complete genotypes of both parents is equal to the mid-parental value. Hence the regression of child on mid-parent is linear with unit slope, from which it follows that the parent-child correlation is $\frac{1}{2}(1+\mu)$.

Vetta claims that Fisher's formulae cannot be true in general because they predict that the parent-child correlation will in some circumstances exceed the sib-sib correlation; Vetta considers this to be impossible, but has given no reason to support this assertion.

I conclude that Vetta has failed to demonstrate that Fisher's formulae are incorrect, and that the alternative form-

ulae presented without proof in his paper are almost certainly incorrect.

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VETTA REPLIES—I consider¹ that the assumptions on which Fisher² derived his formulae are not valid for his model and Bulmer³ raises no objection to this. His objections concern the parent-child correlations not being a linear function of μ , the applicability of the model to disassortative mating and the excess of sib correlation over parent-child correlation. I consider these in turn.

Bulmer³ claims that there is nothing in Fisher's model which restricts μ and that it "may meaningfully take any value between -1 and $+1$ ". Unfortunately, the constraints of Fisher's model are not well understood. In fact, A ($= \mu$ in Bulmer's example) cannot be increased to 1 without limit. For example, when $A \cong 1$, the increase in additive variance is infinitely large. It is difficult to conceive of any trait with infinite variance. That assortative mating could increase genetic variance to infinity is beyond belief. It is more likely that there are genetic constraints which ensure that the increase in variance brought about by a given degree of assortative mating will not be too large. The notion that A can be increased at will, to 1, is unacceptable.

Is there any reason, apart from the formula $\frac{1}{2}c_1c_2(1+\mu)$, to assume that the parent-child correlation will increase linearly with μ ? I know of none. Indeed, it is not absolutely clear how a non-linear increase, $A\tau^2/(1-A)$ in the additive variance should give rise to a linear increase of exactly $\frac{1}{2}A$ in the parent-child correlation. There is, therefore, no reason to believe that A can take any value between 0 and 1 and that the increase in the parent-child correlation should be exactly $\frac{1}{2}A$.

Are there any data which might indicate that an increase in μ will, necessarily, result in an increase in the parent-child correlation? Data on human populations are scarce but studies⁴ on racehorses, which have been bred assortatively for generations, do not support this assertion.

Fisher² showed that assortative mating (AM) will introduce association between phases of factors of an individual and this will increase the additive variance of the population. Wright⁵ asserted that AM will also introduce correlation be-

tween the additive and dominance deviations of mates. I show that this, indeed, happens in Fisher's model. Fisher, however, did not take account of such correlations. In deriving my formulae, I take account of these and thus, provide a synthesis of Fisher and Wright on AM. I show that AM will (1) increase the association between additive deviations of a parent and his progeny and (2) introduce association between the additive deviations of one and the dominance deviations of the other. These considerations require a correction term to Fisher's formulae. This term, when $c_1 = c_2 = 1$, will be $\frac{1}{2}A^2(A-2)$ for parent-child correlation. Note that when $c_2 = 1, (2)$ is 0 but (1) is not 0.

In Fisher's model A has two meanings: it is the genetic component of μ and it also represents genetic correlation between phases of factors. It occurs in the former sense in Fisher's formulae and in the latter sense in my correction term. Obviously, when the correlation between phases of factors is 0, that is, the population is mating assortatively for the first time, my correction term is 0. I, therefore, assert that Fisher's formula for parent-child correlation, as given by Bulmer, is correct for a population which mates assortatively for the first time and is not correct for his model where the population is in equilibrium under a given degree of AM.

Geneticists^{6,7} distinguish between assortative and disassortative mating because they have different genetical consequences. No polygenic model of disassortative mating is available. If one is constructed on the lines of Fisher's model, it will differ from the latter in three respects. First, there will be negative correlation between genotypes of an individual. Second, the genetic variance will decrease. And third, the correlation between the additive and dominance deviations of mates will be negative. Moreover, the attainment of equilibrium may present certain problems. Even if, for a given μ , all these effects were exactly the same in the two models, the correlational formulae may still not be the same. Fisher² restricts himself to a model which results in "an increase in variance". Later^{8,9} discussing it, he uses assortative mating and homogamy by which he means "the tendency of mating like with like" interchangeably.

I considered the possibility that, for his model, sib correction could be greater than the parent-child correlation but rejected it. Briefly, the reason is that, in presence of partial dominance, it is greater than parent-child correlation for one factor as well as for a large

number of factors¹⁰. This holds for assortative mating when the number of factors is small. There is no reason to believe that the situation is reversed when the number of factors is large. Fisher^{2,9}, himself, believed this to be the case for his model and I agree with him. For, in Fisher's model, where add., dev., dom. and cov. stand for additive, deviation, dominance and covariance respectively, sib covariance = cov.(add. dev., add. dev.) + cov.(add. dev., dom. dev.) + cov.(dom. dev., dom. dev.), and parent-child covariance = cov.(add. dev., add. dev.) + cov.(add. dev., dom. dev.). All covariances on the right of these equations are positive. Mendelian segregation will ensure that cov.(add. dev., add. dev.) and cov.(add. dev., dom. dev.) in the two equations are equal. It is obvious that in presence of dominance, sib covariance will be greater than the parent-child covariance. In absence of dominance they will be equal. Incidentally, I do not assert that it is 'impossible' for parent-child correlation to exceed the sib correlation, only that, in Fisher's model, it should not.

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Cholecystokinin produces bait shyness in rats

BECAUSE an injection of cholecystokinin (CCK) (or of the synthetic C-terminal octapeptide of CCK¹) reduces food intake in the fasted rat, it has been suggested by Gibbs, Smith and colleagues²⁻⁶ that CCK may be a satiety signal. We present here evidence against this conclusion.

To quote from ref 4, "In order to establish CCK as a physiological satiety signal, one requirement is that CCK does not inhibit feeding by inducing nausea or other subclinical sickness". An attempt to detect such sickness was made by injecting CCK after the rats had drunk a saccharin solution. If sickness occurs after CCK then less of the saccharin solution should be drunk on a subsequent occasion^{7,8}.

The tests for nausea already performed^{2,3}, however, were quite insensitive. In the first² the rats were extremely thirsty (23½ h) and were given a very attractive sweet taste to drink (0.25% saccharin). Such test conditions would

only be expected to reveal a very strong nausea induced by CCK. The dose of CCK used in this nausea test suppressed eating by only 50% when the CCK was injected before the rat ate². This suggests that only a mild degree of nausea was induced by that dose of CCK if nausea was indeed the explanation. The results of the test showed no effect of CCK (40 U kg⁻¹) or synthetic terminal octapeptide of CCK (40 U kg⁻¹) but a strong effect of 0.15 M lithium chloride in this dosage is a very powerful aversive agent. Even higher levels of thirst were used in a comparison of apomorphine, another strong aversive agent, with CCK (40 U kg⁻¹) (ref. 4). Conditioned aversion was shown to apomorphine but not to CCK. Whilst these studies show that in the doses used, CCK (or the C-terminal octapeptide) is less aversive than LiCl or apomorphine, more sensitive tests of nausea might reveal intermediate degrees of aversiveness.

In our first experiment we used rats (Charles River, male Sprague-Dawley) 22-h thirsty and given 8-min exposure to a novel neutral flavour (almond Schilling 0.5% in water). Immediately after this drinking period nine rats were injected intraperitoneally with 40 Ivy dog units per kg of the terminal octapeptide in saline⁹, and 8 h later the rats were given water *ad libitum* for 2 d. Then they were again water deprived for 22 h. Both groups were then again presented with the almond solution for 8 min. The mean amount consumed on the first day of exposure was almost identical for the experimental rats (10.3 ml) and for the control rats (10.4 ml), but on the second presentation the control rats drank a mean of 14 ml as against 9.6 ml consumed by experimental animals ($t=3.66$, d.f.=16, $P<0.01$). We used an even more sensitive indicator of conditioned aversion in our second experiment by using a preference instead of a single bottle method as a test. A group of 14 naïve Sprague-Dawley rats underwent 22 h water deprivation; half the rats were then presented with almond flavoured water and the others with banana flavoured water (both 0.5% Schilling). After 24 h of water *ad libitum* the rats were again deprived of water for 22 h and allowed to drink the alternate flavour. Immediately following the consumption of this flavour, the experimental rats were injected with CCK octapeptide as above, while the control rats were injected with vehicle. After 24 h of water *ad libitum*, rats were again fluid deprived for 22 h. They were then given a 30-min simultaneous choice between the two flavoured solutions. The controls drank a mean of 8.9 ml of the flavour presented on the first day, and a mean of 8.6 ml of the fluid presented on

the second day when these fluids were simultaneously presented. In contrast, the rats injected with the CCK C terminal octapeptide drank a mean of 9.4 ml of the flavour that had been presented on the first day, but only a mean of 3.3 ml of the flavour paired with the octapeptide when the two flavours were presented simultaneously. There was a significant difference between the experimental and control groups ($t=4.36$, d.f.=12, $P<0.001$), indicating a larger conditioned aversion due to the octapeptide measured by this more sensitive method. We therefore conclude that the C terminal octapeptide of CCK in the dose administered to produce apparent satiation causes some form of sickness in the rat. Whether CCK is a satiety hormone is therefore left in considerable doubt.

We thank Dr A. Ondetti, Squibb Institute for Medical Research, for CCK octapeptide.

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GIBBS AND SMITH REPLY—The production of a conditioned taste aversion, using a single dose of the synthetic octapeptide of CCK as the unconditioned stimulus, is not a critical test of the hypothesis that CCK mediates all or part of the satiety effect of food in the intestine. One indirect test of the hypothesis using the taste aversion paradigm would be the comparison of dose-response curves for both satiety and taste aversion. If the doses of CCK required for satiety were always equal to or larger than the doses that are aversive enough to produce a conditioned taste aversion, this would be indirect evidence against the hypothesis. Such a comparison has not been performed. The hypothesis will be most critical tested, however, when the concentration of circulating, endogenous CCK can be measured reliably during satiety. Then it should be possible to decide if ingested food releases sufficient CCK to account for the satiating effect of that ingested food.

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reviews

Landscape legacy for Ireland

R. Common

The Irish Landscape. By F. Mitchell. Pp.240+32 plates. (Collins: London, 1976.) £5.50.

As an environmental scientist, and as a countryman at heart, Professor Mitchell is concerned in this book with the interactions of natural phenomena and the relationships between man and environment that are now reflected in the Irish landscape.

The author first provides an outline of the geological evolution of Ireland, emphasising the broad relationships between rock type, rock structure and denudation process in the disposition of the major relief features. Professor Mitchell embellishes his narrative, from the outset, with comments that are definitive or with opinions that are open ended. He uses the map appropriately, to show space relationships, and the diagram effectively, to indicate the time sequences of formative events.

Explanatory descriptions of selected features that are associated with near-glacial and glacial conditions introduce the next chapter, which is concerned with palaeogeographical conditions in the Quaternary era. The author provides an interpretation which enlightens rather than confuses, and resists the temptation to depart from valuable generalisation for the complexities of local detail. His subsequent treatment of the development of Irish soils, plant covers and animal populations as climatic conditions, land and sea distributions changed in post-glacial time is equally thoughtful and disciplined.

Chapter 4 deals with the first farmers and their growing influence on the physical environment over a timespan of almost 4,000 yr. The findings of the archaeologist and the palaeobotanist provide the basis for the author's suggested sequence of landscape changes. Amendments, no doubt, will follow as more evidence becomes available, but these should not diminish the importance of this particular contribution or the diligent research work of fellow scholars.

In dealing with the landscape from early mediaeval times to 1900 the author reinterprets historical events in terms of human activities on the land. The human errors and pressures which encouraged environmental deteriora-

tion in this period seem to have influenced his thoughts on present conditions. Chapter 6, in fact, begins with a review of significant features in the contemporary scene and then considers the options for change which can enrich or degrade them. Understandably, therefore, the final chapter develops this important theme of a landscape legacy which future generations of Irish people will inherit.

This well illustrated book is written with a direct style, a clarity of thought

and a turn of phrase which make for pleasurable reading. It will appeal to those with general interests in Natural History besides satisfying those with particular interests in Ireland. In academic terms it claims a significant place beside the writings of R. L. Praeger, J. K. Charlesworth and E. E. Evans. □

R. Common is Reader in Geography at Queen's University, Belfast, Northern Ireland.

Pheromone communication

Animal Communications by Pheromones. By H. H. Shorey. Pp viii+167. (Academic: New York and London, 1976.) \$16.50; £10.05.

PHEROMONES are chemicals produced by an individual which elicit specific behavioural or developmental reactions when perceived by other individuals of the same species. The presence of pheromones has been deduced in most animals, from Protozoa to primates, and in some groups, such as the insects and mammals, many pheromones have been identified chemically, and the synthetic compounds shown to have the same effects as the natural substances.

A comprehensive survey of the functions of pheromones requires a formidable knowledge of the morphology, physiology, behaviour, and even ecology, of members of almost every animal phylum. The author of *Animal Communication by Pheromones* points out that no single-authored monograph on pheromones has hitherto been published, and that his book is an attempt to review, digest and present in a cohesive manner pheromone communication within the entire animal kingdom. Unfortunately, the book falls far short of these objectives.

Only brief mention is made of 'primer' pheromones, a surprising omission, since the ways in which these substances induce developmental changes in the recipients can usefully be compared in animals as diverse as the insects and the mammals, and discussion of those pheromones which

induce both behavioural and developmental changes must necessarily also be limited. The arrangement of chapters dealing with particular 'classes' of pheromones—recognition, aggregation, dispersion, and so on, inevitably leads to repetition. The author's style is turgid and often leads to confusion. For example, what is one to make of: "Colonization begins with the alighting on a suitable host tree of an initially invading beetle, which may be a male or female (*but not both*), depending on the species" (p56) (*my italics*).

Many of the line drawings are unnecessary, and most of the plates are too dark and without contrast. Figure 14 (courtesy of N. W. Nowell) has a hopelessly inadequate legend, and must be meaningless to all but Dr Nowell, even with reference to the text. And why did the publishers decide to begin each chapter on an odd-numbered page? This may be useful in multi-authored compendia, where each contributor is normally given a number of reprints, but in the present volume it results in some chapters ending with up to one and three quarters blank pages. Since £10.05 buys only 121 numbered text pages, these blank spaces are expensive. The book contains an extensive bibliography of 726 titles, together with taxonomic and subject indexes. It cannot be recommended.

K. C. Highnam

K. C. Highnam is Reader in the Department of Zoology at the University of Sheffield, UK.

Plants and pathogens

Plant Pathosystems. R. A. Robinson. Pp. x+184 (Springer: Berlin, Heidelberg and New York, 1976.) DM48; \$19.70.

MR ROBINSON is a plant pathologist who has been much involved over many years in breeding crop plants for resistance to important pathogens in a number of developing countries. It is therefore not surprising when he states in his preface that this book was written in remote places far from good library facilities. This being so, one asks what would have been the difference had this been otherwise, because *Plant Pathosystems* is already a very good book and unique in many ways. The text is based on long and varied experience of field plant pathology in Africa and essentially is a series of perceptive analyses of interactions between populations of host plants and populations of pathogens, and of assessments of what such analyses teach about the management of crops so as to decrease losses caused by plant pathogens to economically low levels and, in agricultural terms, more or less permanently.

The book begins with a short account of the systems concept and of the properties, analysis and management of systems. This is useful and interesting to the uninitiated such as the reviewer. The main substance of the book starts with chapter 2, which first describes plant pathosystems, defined as any subsystem of an ecosystem which involves parasitism. It then characterises the "esodemic" and the "exodemic" as parts of epidemics that depend, respectively, on infection from within (auto-), and from outside (allo-) crops and which are therefore of quite different significance in the development of epidemics especially in relation to the type of disease resistance that controls infection.

Chapters 3-6, as the core of the book, deal in depth with analysis and management of pathosystems comprising either interactions between vertical resistance and vertical pathogenicity, or between horizontal resistance and horizontal pathogenicity. Subjects discussed include the properties of vertical and horizontal resistance in genetical and epidemiological terms, the nature and role in disease of gene-for-gene relationships and of strong genes, genetic flexibility of host and pathogen in relation to the origin and developments of epidemics, and the paramount importance of horizontal resistance in crop management both generally and as illustrated by many well-chosen examples of important diseases of crops of different types, and especially of maize and sugar-cane.

Chapter 7 is concerned with the operation of some of these factors in polyphyletic crops, again illustrated with examples, and chapter 8 is a short review of the nature and assessment of the vulnerability of crops to serious damage by pathogens, and how vulnerability can be decreased by various measures which prevent, or prepare for, the exposure of crops to virulent pathogens.

Chapter 8 contains a series of conclusions which emphasise the value of the systems concept and of well conceived programs of domestication of crop plants which exploit the superior qualities of horizontal resistance. A last chapter on terminology defines or describes, often at some length, no fewer than about 300 words, terms and concepts in some 24 pages. This is a particularly valuable and essential part of the book. There are about 100 references, a small number in relation to the scope of the book; even so, a few are unexpected. The shortness of the list is explained by the circumstances and style in which the book was written. In contrast, there is an extensive index of about 1,000 entries.

Mr Robinson states in his Preface that his book is theoretical and speculative. But much of it is a good deal more than theoretical, as would be expected from Mr Robinson's background; and the speculation is reasoned and not unrealistic. Quite a lot of the book is controversial. Thus, Mr Robin-

son's concept of vertical and horizontal resistance will be regarded as oversimplified by plant pathologists who have studied resistance at the cellular and molecular levels. Also, Mr Robinson does not like the extensive use of vertical resistance in plant breeding and says so very plainly. But he is an enthusiast for the use of horizontal resistance whenever possible and that, in his opinion, should be almost always.

Some plant breeders will not agree with the extremities of his approach. The writing from time to time is forthright and provocative but attractively so and certainly should not offend those against which it is sometimes quite pointedly directed. The book is well and clearly written but must be taken slowly because almost each paragraph on each page contains one or more substantial points which must be understood if the main themes which are propounded are to have their full impact. But the effort called for is very well rewarded because few books published on plant pathology during the past three decades are so stimulating, challenging, and have been written with such worthy objectives. *Plant Pathosystems* will undoubtedly cause quite a stir among plant pathologists and plant breeders, for most of whom it must be regarded as compulsory reading.

R. K. S. Wood

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Laser physics

Principles of Lasers. By O. Svelto. Pp. x+376. (Plenum: New York and London, 1976.) \$30.00.

THERE is light, and light. Or as Svelto might put it, the properties and quality of radiation can be defined and quantified by the first, and higher-order, correlation functions of the electromagnetic field. Thus, to first-order the coherence of laser light is revealed in its exceptional brightness and monochromaticity. The unique spatial and temporal coherence of such sources has already led to a number of practical applications, and largely accounts for the current scientific interest in this subject.

This book develops the theory and techniques of laser physics from first principles (quantum mechanics and electromagnetism), is completely self-contained, and should prove of particular value to the undergraduate reader. The text is well illustrated, very carefully referenced, and includes imaginatively chosen questions at the close of each of its nine chapters. (Problems in the introductory chapters,

for example, range from black-body radiation to practical design problems of ruby, dye, He-Ne and CO₂ lasers.) Pumping processes, passive optical resonators, continuous and transient energy extraction (including the semi-classical treatment of π pulses, photon echoes and passive-mode-locking) are treated in detail; illustrative applications, including holography and optical data-processing, are treated more generally. Topics involving quantisation of the electromagnetic field (such as laser noise) are not discussed.

In my opinion, unstable optical resonators warranted a more extensive discussion: for example, the importance of diffuse apertures was not considered, and Anan'ev's work was not referenced. Surprisingly, the highly efficient CO laser and recent Kr₂* and Ar₂* excimer (and halide exciplex) lasers receive no mention. These are, however, minor criticisms of a book which has been carefully translated and which makes excellent reading.

Ian J. Spalding

Ian Spalding works on laser applications at the UKAEA's Culham Laboratory, Abingdon, UK.

Calcium in control

Calcium in Biological Systems. (Symposia of the Society for Experimental Biology.) Edited by C. J. Duncan. Pp. viii+485. (Cambridge University: Cambridge, London and New York, 1976.) £18.

THE study of extracellular calcium metabolism and its hormonal control has progressed steadily over many years. Interest in intra-cellular roles of calcium, confined for some time to its regulatory function in muscle, has recently grown rapidly following independent advances in several areas. Calcium is now known to be a key factor in triggering secretion, activating cells for division, controlling inter-cellular communication and regulating glycolysis and respiration, in addition to its involvement in contractile systems and in nervous conduction.

This latest Symposium of the Society for Experimental Biology considers mainly these intracellular systems. The organisers have concentrated attention on the general problems of how intracellular calcium levels are controlled, rather than on the detailed analysis of the effector roles of Ca^{2+} which differ greatly in different systems. This is a particularly difficult experimental field for the very reason that calcium is such a good controlling ion; its intracellular concentration is normally in the submicromolar range so that small fluxes have large effects.

It is only within the last five or ten years that the discovery of aequorin by Shimomura and Johnson has provided a direct approach to its measurement. These authors contribute a useful paper on the properties of this interesting luminescent protein and other papers by P. F. Baker and C. C. Ashley *et al.* describe its application in nerve axons and in muscle fibres. Baker also provides a good general analysis of intracellular Ca^{2+} regulation, and Ashley *et al.* consider the technical problems in the quantitative interpretation of aequorin luminescence. They also describe the use of a microscintillator probe to follow the influx of $^{45}\text{Ca}^{2+}$ but they do not evaluate the relative merits of these two techniques.

Two good introductory chapters deal with the general chemistry of Ca^{2+} and its complexes (R. J. P. Williams) and with a variety of natural and synthetic ionophores for divalent cations (M. Truter). I missed a similar introductory section on the use of electrophysiological techniques and concepts and the uninitiated will find unfamiliar jargon and usage in this field. For example, the significance of a poten-

tially interesting paper on the effects of intracellular calcium on membrane permeability (R. W. Meech) was obscured by the use of the term 'activation' in different and undefined senses.

Almost half of the twenty papers concern contractile systems and three of these were on troponin. A clear account of its evolutionary relationships with parvalbumins and myosin light chains, based on amino acid sequences (J. H. Collins), was complemented by a comparison of control functions in different species and a study of the functional interchangeability of these regulatory proteins *in vitro* (A. Szent-Gyorgyi). It was pointed out (S. Ebashi *et al.*) that in spite of such homologies one must be cautious about arguing too closely from the regulatory mechanisms in skeletal muscle to other situations, since in smooth muscle, for example, calcium behaves as an activator rather than as a derepressor.

Several papers considered the problems of coupling between excitation and contraction. In skeletal muscle (S. R. Taylor and E. Godt) a depolarisation induced release from sarcoplasmic reticulum is the main physiological mechanism. The more complex situation in heart muscle is reviewed at the physiological level by R. Niedergerke

et al., and some of the biochemical problems are discussed by E. Carafoli and M. Crompton in their critical evaluation of the role of mitochondria in Ca^{2+} regulation.

One conclusion to be drawn from several of the contributions is that with the exception of sarcoplasmic reticulum and of Ca^{2+} uptake by mitochondria, little is known about the nature of intracellular calcium stores and their control. The large reservoir of calcium in the nucleus can best be described as a pool of ignorance.

The conference is ably summarised in a concluding chapter (A. Weber) which also mentions relevant points from the discussion. These tantalising glimpses make one wish that a more extensive record of this part of the meeting could have been included in the book. Nevertheless, the account as it stands provides a valuable survey of a diversifying and developing subject. It will be particularly valuable to workers in the calcium field who wish to keep up-to-date with advances in less familiar parts of their subject.

N. Michael Green

N. Michael Green is a member of the MRC staff, working at the National Institute for Medical Research, London, UK.

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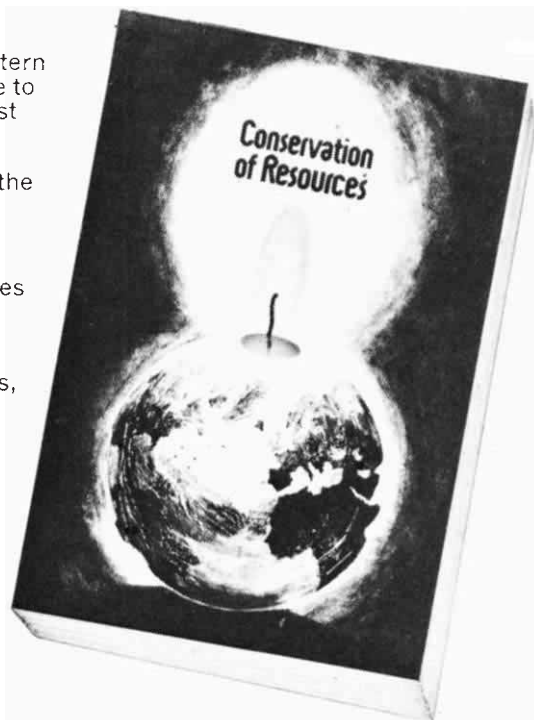
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Pragmatics

Language and Context: The Acquisition of Pragmatics. (Language, Thought and Culture: Advances in the Study of Cognition.) By E. Bates. Pp. xiv+375. (Academic: New York and London, 1976.) \$19.95; £12.20.

PSYCHOLINGUISTICS has traditionally depended on linguistics proper to supply research topics. When linguists produce a rule to which the sentences of our native language can conform, psycholinguists can then go off and try to explain either the sense in which we "know" this rule, or how we manage to act as if we knew it. Until quite recently, the rules in question were almost exclusively rules of syntax, and the psycholinguist's problem was, for instance, to explain the mental organisation that might lead us to exclaim "Ah, there's the book that I was hunting for!" but never "Ah, there's the book that I was wondering where was!"

In the past few years, however, linguists have been giving increasing attention to the study, under the heading of "pragmatics", of the conventions surrounding the use of various linguistic forms. For instance, "Can you shut the window" has a syntactic form normally associated with questions, yet it might easily function as a request. Furthermore, it is a more

polite request than a simple "Shut the window". In some circumstances, "Do you think it's a bit chilly in here?" might be more polite still, although at other times it will just be annoyingly indirect.

The psycholinguist Elizabeth Bates has set out to chart the mastery by children of these, and other, subtleties of linguistic usage. Working from studies of pre-school age Italian children, she marks the onset of such phenomena as talking about talking, laying emphasis on particular words, discussing situations beyond the here and now, and wording an appeal for a sweet so as to make it as persuasive as possible. She also considers, from the standpoint of Piaget's theory of cognitive development, what mental capacities are required to reach these pragmatic milestones, and so sets linguistic development in the context of general cognitive development.

Professor Bates has written an excellent and fascinating book. She is considerate to the reader, explaining clearly and carefully both the theoretical background, and the design and interpretation of her experimental studies. It is a book pregnant with possibilities for further research, and one can only hope that others who follow her, maintain her standards of liveliness and clarity. **S. D. Isard**

S. D. Isard is Lecturer in Experimental Psychology at the University of Sussex, UK.

Soils of arid regions

Soils of the Arid Regions. (Developments in Soil Science 6.) By H. E. Dregne. Pp. xii+237. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 75.00; \$28.95.

THE aim of this book in attempting to collect together the available information on arid region soils is highly commendable. In attempting to cover such a vast topic in only 223 pages, however, the author sets himself an impossible task. The inevitable result is a book which is too specialised for the layman and too superficial for the professional pedologist.

From a brief introduction to arid regions (excluding polar deserts) the author moves on to consider with little comment, various approaches to the classification of arid region soils, finally selecting the USDA 7A scheme as a basis from which to work. Chapter three consists of a short discussion of each of the soil orders found in arid regions together with a cursory summary of the geomorphology of arid regions. In the following six chapters the distribution of arid soils is considered on a continental basis; accompanying each of these chapters is an absurdly small scale map showing the distribution of the principal associations of great soil groups. The choice of symbols is such that on several maps it is impossible to differentiate between alfisol, aridisol and mollisol groupings. This approach to the consideration of soil distribution leads to a mass of needlessly repetitive information on each great group, which could, together with better maps, have been condensed into one chapter. The last three chapters cover very briefly those chemical physical and biological properties of soils which are (according to the author) important for management. Throughout the whole text discussion on pedogenesis is extremely limited and very superficial.

The standard of the twenty-six line illustrations (there are no photographs) is extremely low: several are illegible and many others are irrelevant, adding nothing to the text.

Despite all these shortcomings, had the book been reasonably priced it could have been recommended, simply because it is the first text to attempt to draw together information on arid region soils. With its outrageous price of about £19.00, brief coverage and appalling illustrations, however, there is no reason to recommend this book. Sadly there still remains a great need for a comprehensive text on the soils of arid regions. **M. J. Alexander**

M. J. Alexander is Lecturer in Geography at the University of Durham, UK.

Air pollution

Effects of Air Pollutants on Plants. (Society for Experimental Biology, Seminar Series 1.) Edited by T. A. Mansfield. Pp. 209. (Cambridge University: Cambridge, London and New York, September 1976.) Hardcover £8.50; paperback £3.80.

THIS volume appears 15 months after the seminar on which it is based. The authors have concentrated on their own findings, and consequently much material appears here for the first time; coverage is uneven and references highly selective. Less understandably, there is no attempt to place the contributions in a wider framework or to define the outstanding problems. For example, the chapter on uptake of pollutants (Unsworth, Biscoe and Black) mentions only the resistance analogy approach; such work surely should form the basis for simulation modelling of the growth of polluted crops, but nowhere in the book is this suggested. Only Bradshaw on evolution and pollution gives an integrated account of his field. The central challenge of bridging laboratory and

field observations is raised both for fluorine (Davison and Blakemore) and for ozone (Manning and Feder)—the latter authors, like most Americans, eschew growth analysis. Both of these chapters find contradictions between laboratory and field data: could the method of expressing pollutant levels (as mean, peaks or dose) be partly responsible? The singular properties of open top chambers (McCune, Maclean and Schneider) make it unlikely that this type of enclosure will yield unambiguous results. By recording changes in enzyme activities following fumigations, the Lancaster group point the way to pollutant-specific physiological tests. Would that more work on SO₂ and lichens were as analytical as that described here (Nieboer, Richardson, Puckett and Tomassini). Two useful appendices summarise data on air chemistry and references to biochemical effects. Overall, we are given valuable data but little perspective; a book for the specialist.

J. F. Farrar

J. F. Farrar is Lecturer in the Department of Botany at the University of Liverpool, UK.

obituary

Professor Philip Macdonald Sheppard, FRS, died on October 17, 1976. He was an outstanding scientist, an inspiring teacher, a formidable adversary and a very good friend.

Philip Sheppard was born in 1921, the son of a schoolmaster. He was educated at Marlborough College. He joined the Royal Air Force Volunteer Reserve in 1940, and was a prisoner-of-war from 1942 to 1945. Immediately after the war he went up to Worcester College, Oxford, to read Zoology, and in 1948 was awarded a second class Honours Degree. It is said that he could easily have gained a 'first' had it not been for his single-minded devotion to genetics, the subject to which he dedicated the rest of his life.

He studied for the D.Phil. at Oxford under Dr (now Professor) E. B. Ford, and obtained the degree in 1951. From 1951 to 1956 he was Junior Research Officer in the Department of Zoology, taking a year off in 1954 to work with Professor Theodosius Dobzhansky at Columbia University. In 1956 he was appointed Senior Lecturer in Genetics in the Department of Zoology at Liverpool University. He was promoted to Reader in 1959. In 1963 he became the first Professor of Genetics at Liverpool, and remained in this post until his death. He was elected to a Fellowship of the Royal Society in 1965, and to an Honorary Fellowship of the Royal College of Physicians in 1975. In 1974 he was awarded the Darwin Medal of the Royal Society, and in 1975 the Gold Medal of the Linnean Society.

The bare description of a distinguished career shows little of the great impact that Philip Sheppard made on the study of ecological genetics. He had an almost uncanny ability to see the next step in an argument, the next experiment to be done. He applied this ability freely and generously to the work of others, and generated many an idea or experiment that came to be published under another name.

His own work completed the transformation of ecological genetics from a science of observation to a science of experiment. His central interest lay in the mechanics of natural selection and its effects on the genetic constitution of organisms. This interest guided his work with Sir Ronald Fisher and Professor E. B. Ford on polymorphism

in the moth *Panaxia dominula*, in which they finally demonstrated that the temporal changes or gene frequency were the consequences of natural selection. It motivated his studies with Professor A. J. Cain on polymorphism in the snail *Cepaea nemoralis*, studies which showed that spatial variations in this species were also due to selection, and which culminated in his beautiful experiments on selective predation by thrushes. It also guided the work with Sir Cyril Clarke on mimicry in butterflies of the genus *Papilio*, demonstrating the evolution of 'supergenes', linked polymorphic complexes of genes maintained in a state of linkage disequilibrium by strong selective interactions. Their interest in such interactions led Sheppard and Clarke to studies of human blood groups, bringing them finally to a method of preventing Rhesus haemolytic disease. Apart from these classic experiments, Sheppard also made important studies on the evolution of dominance, the genetics of industrial melanism in moths, heavy metal tolerance in plants, insecticide resistance in mosquitoes, and schizophrenia, anencephaly and spina bifida in man. In 1958 he published *Natural Selection and Heredity*, and the fourth edition was issued shortly before his death. It is still the best introductory book about ecological genetics, and one of the rare breed of elementary texts that are quoted in the research literature because of the original ideas that they contain.

He was an exceptional teacher. His transparent intellectual honesty, his passion for truth and rigour, and perhaps above all his patent desire that his hearers should understand and enjoy his subject, made an enduring impression on his students. As a colleague and friend he was a delight, and a very good companion. His comments on cherished ideas or manuscripts were sometimes blunt to the point of pain, but they were always given with good humour, never tinged with malice. His friends said that he reversed the normal academic procedure; he stabbed you in the front, and then did you favours by stealth. Hating pomposity, he was marvellously uncorrupted by success. As a distinguished Darwin Medallist he was much the same Philip Sheppard as the Junior Research Officer at Oxford.

He bore his three-year illness with quite extraordinary courage and cheerfulness.

His life can be epitomized in the words of one who was also a prisoner-of-war: "To serve his vision, to protect it against all plausible substitutes, reasonable approximations and coward compromises is still, I believe, the knightly duty of contemporary man. He has only to remain steadfast in pursuit of it and his life will achieve something which is greater than happiness and unhappiness: and that is meaning." **B.C.C.**

Sigurd Zienau died on October 18, 1976 at the early age of 55 years. He had been a Reader in Physics at University College London since 1965, following a period from 1954 as a Lecturer, and as ICI Research Fellow at Liverpool.

Dr Zienau was trained in the old school of European physics, being a student of Heitler, Pauli and Fröhlich, after a distinguished period as a mathematics undergraduate at Birkbeck College.

His name is familiar to solid state physicists as a co-author of the theory of the polaron through which, in 1950, field theory was introduced into solid state physics. He will also be remembered in the worldwide community of theoretical physicists for his important revisions of two classic texts in theory: both Heitler's *Quantum Theory of Radiation* (3rd ed.) and Mott and Massey's *The Theory of Atomic Collisions* (3rd ed.) owe much to his labour and insight. He had a phenomenal memory for the literature of theoretical physics and would recall from obscure journals published in the first half of the century the seminal work that could shed light on modern problems. His main research interests lay in scattering theory and electrodynamics, but he had a scholarly interest in a much wider range of subjects.

Dr Zienau's published papers do not, in quantity, do justice to his gifts. However, they are thoughtful contributions and each one represents the distillation of many months hard work, as well as hard argument, with his co-workers. There are three phases of his research represented in these papers. In the early 50s he published work on dielectrics, especially on the motion of

slow electrons in polar materials. Later in the 50s and early 60s he published a series of six papers on electro-dynamics including contributions to damping processes, line shapes, inter-molecular forces (including retardation and three body interactions) and gauge problems. The final set of papers concern various specialised aspects of field theory.

He trained about ten research students who now teach throughout the world and these will always have that special magic that comes from having worked with and learned from Sigurd.

In recent years he interested himself in the history and philosophy of science and was active in the British Society for Social Responsibility in Science.

He will be sadly missed by his friends, colleagues and students at University College. He leaves a young daughter and son by his second marriage, and one son by his first.

E. A. Power

F. F. Heymann

J. V. Peive, the Soviet soil scientist, died after a long illness on September 13, 1976, aged 70. As well as being one of the leading Soviet agricultural scientists of the post-war era, Dr Peive had been politically active and, according to Tass, his official obituary was signed by Mr Brezhnev and other prominent Soviet politicians and scientists. Of Latvian extraction, he was born in 1906 in the adjoining Toropets district of Russia and, after training as a teacher, graduated in 1929 from the Timiryazev Agricultural Academy in Moscow.

For 13 years from 1931 he worked in the All-Union Flax Research Institute in Torzhok, some 200 km north-east of Moscow, rising to the position of Director. After the war he became Rector of the Latvian Agricultural Academy in Riga, carrying out his own specialist investigations in the Laboratory of Biochemistry of Soils and Trace Elements in the Institute of Biology. He published a text-book on the biochemistry of soils in 1961 but will be best remembered for his work on the effects of trace elements in soils on plants and animals. Several volumes of the Transactions of his laboratory, dealing with different aspects of trace element problems, have been published by the Latvian Academy of Sciences, and he chaired the Co-ordinating Commission on Trace Elements in Soils, Plants, Animals and Human Nutrition in the USSR. Dr Peive's wider interests in soils were recognised by his appointment as Editor-in-chief of *Pochvovedenie*, the Soviet journal of soil science, a post that he had occupied for the past 14 years.

Peive rose to the Presidency of the Latvian Academy of Sciences and, after 13 years as a Corresponding Member, was elected an Academician of the Academy of Sciences of the USSR in 1966, in which he held the post of General Secretary. He was Prime Minister of Latvia from 1959 until 1962 and was one of the three Latvian representatives in the Soviet of Nationalities, one of the two Chambers of the Supreme Soviet of the USSR, and Chairman between 1958 and 1966.

He seldom attended scientific gatherings outside Russia, although only ill-health prevented his participation in the meeting of Commissions II and IV of the International Society of Soil Science in Aberdeen in 1966. In private discussion he talked about his work with much less formality than is the case with many Soviet scientists and often introduced a light-hearted note. His major contribution to Soviet agriculture was in the immediate post-war years when he pointed out the existence of trace element problems in many Russian soils, particularly deficiencies of boron, zinc, manganese, cobalt, copper and molybdenum.

R. L. Mitchell

Professor Leo Pincherle died suddenly on October 25, 1976. I came to know him well during the last years of his life. He regularly attended the Bedford/Westfield College solid state physics seminars. Characteristically, he spoke little at these meetings, but his rare contributions were profound and to the point. He was modest to a fault and abhorred the limelight; yet his colleagues, friends and students know of the innumerable unobtrusive ways in which he served the cause of scholarship in teaching and research.

Leo Pincherle was born in Bologna in 1910. He came from a line of distinguished academics—the mathematician Salvatore Pincherle was a grandfather—but Leo was the first of the family to take up physics as a career. His first publication (1931) reported a calculation of the wavelength of the oscillations in Hull's magnetron; and the interest in the theoretical principles underlying practical devices marked his work to the end. He was with Fermi in Rome, later moved to Padua and came to England shortly before the Second World War as a refugee from Mussolini's Italy. During the difficult years which followed he was befriended by the late H. T. Flint who brought him as a lecturer to King's College, London. Leo's early research, on a variety of topics in spectroscopy, atomic physics and electromagnetic theory, demonstrates the width of his scientific interests. But his great productive period

came at the Telecommunications Research Establishment (TRE), Malvern, where he was Principal Scientific Officer from 1948 until 1955. Here he established himself as a leading authority on energy band theory. The 1953 paper on the band structure of lead sulphide (Bell *et al.*), in which the cellular method was first applied to a diatomic semiconductor, is an early classic in the field, and fundamental theoretical work on applications of group theory appeared in the form of TRE Memoranda. Pincherle returned to university life in 1955 when he rejoined Flint, by then head of the physics department at Bedford College, London, as a University Reader; overdue promotion to Professor of Mathematical Physics came in 1969. A fruitful collaboration with P. M. Lee led to further band structure papers in the early 1960s. In the last ten years of his life Leo Pincherle published few research papers. But he continued to be active and fertile in the many other ways a scholar exerts his influence: as supervisor of research students, as a sought-after contributor to summer schools in Italy, as UK editor of the international journal *Solid State Electronics*, and not least as author of the definitive textbook *Electronic Energy Bands in Solids* (1971) which itself contains much original work. Above all Leo Pincherle loved teaching and the contact with his students, a love which was fully returned, and the prospect of retirement from this activity filled him with dismay. Leo's lectures and publications all bear the stamp of his characteristic approach to physics: careful preparation, great clarity and economy of style, the use of the simplest mathematical techniques appropriate to the physics of the problem in hand. He tackled difficult problems but was never a narrow specialist.

The picture of Leo Pincherle the scientist cannot be separated from that of Leo Pincherle the man. He was devoted to his family, for whom his too-early death is a tragic loss. His sense of humour, dry and delicious, showed in his fund of stories about well-known contemporaries — always amusing and never malicious. His quiet demeanour concealed plenty of fire and imagination, as you soon discovered when you partnered him at bridge. He was the most knowledgeable of men on fundamental aspects of European culture and history and wore his learning lightly; his love of music and the arts was intense, his taste discriminating. He was, in the best sense, both very Italian and very English, combining in his person that internationalism in science and that European spirit which it is so necessary to keep alive today. His friends miss him deeply.

Ernst Sondheimer

announcements

Awards

The Lomonosov medals—the highest award of the Soviet Academy of Sciences—for 1976 have been awarded to **Academician S. I. Vol'fkovich** of the Soviet Academy of Sciences for his work on the chemistry of phosphorous, and to **Dr Hermann Klare**, President of the Academy of Sciences of the German Democratic Republic, for his work on artificial fibres.

The Royal Astronomical Society Gold Medal has been awarded to **Mr John Bolton**, a Chief Research Scientist with the CSIRO Division of Radiophysics at Parkes, NSW, for outstanding contributions to both radio and optical astronomy, particularly for his studies of radio sources in space.

The Clough Medal for 1976–77 has been awarded to **Professor T. S. Westoll**, FRS, Department of Geology, University of Newcastle upon Tyne for contributions to geology, in particular studies of fossil vertebrates and their application to the stratigraphy of the Old Red Sandstone of Northern Scotland. The Clough Award for 1976–77 has been conferred on **Dr E. N. K. Clarkson** of the Grant Institute of Geology, University of Edinburgh, for his work in palaeontology and palaeoecology, in particular on the eyes of trilobites and including trilobites from the Lower Palaeozoic rocks of Scotland.

The Faraday Medal has been awarded to the Executive Director-General of the CERN Laboratories in Geneva, **J. B. Adams**, for his contribution to the design and construction of high-energy particle accelerators.

Meetings

14 March, **Ion Implanted Semiconductor Devices**, London (Institution of Electrical Engineers, Savoy Place, London WC2).

15 March, **Vehicle Navigation and Identification**, London (Institution of Electrical Engineers, Savoy Place, London WC2).

26–28 March, **Changing Sea Bird Populations Of the North Atlantic**, Aberdeen (G. Sarle, Royal Society for the Protection of Birds, Sandy, Beds.).

29 March–1 April, **Submillimetre Waves and their Applications**, Surrey (Institute of Physics, 47 Belgrave Square, London SW1).

5–7 April, **Energy in the 80s**, Middlesbrough (Institution of Chemical Engineers, 165–171 Railway Terrace, Rugby).

18–20 April, **Advances in Extractive Metallurgy**, London (Institution of Mining and Metallurgy, 44 Portland Place, London W1).

19–20 April, **Strain Rate Method of Stress Corrosion Testing**, Newcastle upon Tyne (Society of Chemical Industry, 14 Belgrave Square, London SW1).

Person to Person

The Einstein Archive is at work on a Collected Edition of the Papers of Albert Einstein. Would anyone in possession of, or knowing the whereabouts of, original manuscripts, letters to or from Professor Einstein, or documents or letters mentioning him, of which they are not sure that the Archive already has copies, please contact The Einstein Archive, Institute for Advanced Study, Princeton, N.J. 08540.

Exchange large furnished house Berkeley for one in London, July 1977 to August 1978. Pleasant neighbourhood, near schools and public transport and within walking distance of the University of California (1 mile). Transportation to San Francisco very convenient. Exchange of car (Saab) also considered. Please contact: Dr Richard Malkin, 1967 El Dorado, Berkeley, California 94707, U.S.A.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

9–11 May, **Hepatotropic Factors**, London (Ciba, 41 Portland Place, London W1).

17 May, **The Changing Face of Cancer**, London (Marie Curie Memorial Foundation, 124 Sloane Street, London SW1).

22–25 May, **Autoradiography in Pharmacology and Toxicology**, Uppsala (S. Ullberg, University of Uppsala Biomedical Center, Box 573, S-751 23, Uppsala, Sweden).

31 May–2 June, **Lasers in Chemistry**, London (M. West, The Royal Institution, 21 Albemarle Street, London W1).

1–2 June, **30th Chemists' Conference**, Scarborough (K. Speight, BSC, Sheffield Laboratories, Hoyle Street, Sheffield).

15–26 August, **Scientific and Mathematical Foundations of Engineering Acoustics**, Cambridge, Mass. (MIT, Room 3-366, Cambridge, Mass. 02139).

31 August–2 September, **Cell-Cell Recognition**, Oxford (A. S. G. Curtis, Cell Biology, University of Glasgow, UK).

4–8 September, **Low Energy Ion Beams**, Salford (Institute of Physics, 47 Belgrave Square, London SW1).

10 September, **The Microscopic Structure and Dynamics of Liquids**, Corsica (J. Dupuy, Physics, University of Lyons, 69621 Villeurbanne, France).

13–16 September, **Scintillation Counting**, Bath (The Secretary, Analytical Division, Chemical Society, Burlington House, London W1).

26–28 September, **On-line Surveillance and Monitoring of Process Plant**, London (E. Wakely, Society of Chemical Industry, 14 Belgrave Square, London SW1).

2–4 October, **5th Biennial Symposium on Turbulence**, Rolla, Missouri (J. L. Zakin, Chemical Engineering, University of Missouri, Rolla, Missouri 65401).

10 October, **The Display of Nondestructive Testing Data**, London (Institute of Physics, 47 Belgrave Square, London SW1).

19–21 October, **Current Topics in Drug Research**, Uppsala (R. Dahlbom, Biomedical Center, University of Uppsala, Box 574, S-751 23, Uppsala, Sweden).

9–11 November, **Atherosclerosis**, Milan (Fondazione G. Lorenzini, Via Montenaполеone 23, Milano, Italy).

28 November–2 December, **Protons and Ions Involved in Fast Dynamic Phenomena**, Paris (C. Troyanowsky, 10 rue Vauquelin, 75231 Paris Cedex 05, France).

23–28 February 1978, **5th International Wheat Genetics Symposium**, New Delhi (M. V. Rao, Cummings Laboratory, Indian Agricultural Research Institute, New Delhi-110012, India).

10–14 April, **9th International Symposium on Carbohydrate Chemistry**, London (J. F. Gibson, The Chemical Society, Burlington House, London W1).

12–14 April, **4th European Immunology Meeting**, Budapest (The Secretariat, c/o MOTESZ, H-1361 Budapest, Hungary).

17–20 April, **10th National Atomic and Molecular Physics Conference**, London

(Institute of Physics, 47 Belgrave Square, London SW1).
 20–25 August, **3rd European Conference on Analytical Chemistry**, Dublin (Euroanalysis III, Institute for Industrial Research and Standards, Ballymun Road, Dublin 9, Ireland).
 4–7 July, **7th International Cryogenic Engineering Conference and Exhibition**, London (Institute of Physics, 47 Belgrave Square, London SW1).
 20–25 August, **Plant Cell and Tissue Culture**, Calgary (IAPTC Congress, University of Calgary, Calgary, Alberta, Canada).
 21–25 August, **4th International Congress of Cybernetics and Systems**, Amsterdam (J. Rose, c/o College of Technology, Blackburn, UK).
 4–8 September, **4th International Symposium on Physical Organic Chemistry**, York (J. F. Gibson, The Chemical Society, Burlington House, London W1).
 11–15 September, **Thin Film Properties in Relation to Structure**, Loughborough (Institute of Physics, 47 Belgrave Square, London SW1).

Reports and Publications

Other countries—January

Office de la Recherche Scientifique et Technique Outre-Mer, Paris. *Mémoires ORSTOM*. No. 75: Organisation Evolutive d'Un Groupe Agamique—La Section des Maximaux du Genre *Panicum* (Graminées). Par Jean Pernes. 40 francs. Pp. 106. No. 77: Polymorphisme et Modes de Reproduction dans la Section des Maximaux du Genre *Panicum* (Graminées) en Afrique. Par Daniel Combes. Pp. 99. 40 francs. (Paris et Bondy: Office de la Recherche Scientifique et Technique Outre-Mer, 1975.) [101]
 United States Department of the Interior: Geological Survey. Bulletin 1419: Annotated Bibliography of the Geology of Selenium, 1958–74. By Carol A. Gent. Pp. iii+49. (Washington, DC: US Government Printing Office, 1976.) [111]
 Alberta Research Council. Report 75-4: Deep Cretaceous Coal Resources of the Alberta Plains. By J. R. Yurko. Pp. 47. (Edmonton: Alberta Research Council, 1976.) [111]
 Australia: Commonwealth Scientific and Industrial Research Organisation. Division of Soils. Technical Paper No. 30: Some Properties of Red, Yellow and Grey Massive Earths in North Queensland. By R. F. Isbell and G. McL. Smith. Pp. 52. (Melbourne: CSIRO, 1976.) [121]
 United States Department of Agriculture. Index-Catalogue of Medical and Veterinary Zoology. Supplement 20, Part 7: Parasite-Subject Catalogue—Hosts. By D. A. Tolson, M. W. Hood, J. H. Shaw, J. D. Rayburn and S. J. Edwards. Pp. xv+390. (Washington, DC: US Government Printing Office, 1976.) [121]
 Annals of the South African Museum, Vol. 72, Part 1: A New Species of *Bradydium* (Copepoda, Calanoida) from the Mzanza Estuary, Pondoland, South Africa, and a Review of the Closely Related Genus *Pseudotharyx*. By Janet N. Bradford. Pp. 1–10. (Cape Town: South African Museum, 1976.) R.1.70. [121]
 New Zealand: The National Hydatids Council. 16th Annual Report year ended 31 March 1976. Pp. 24. (Wellington: The National Hydatids Council, Ministry of Agriculture and Fisheries, 1976.) [121]
 Smithsonian Contributions to Zoology, No. 242: Studies in Larval Amphibian Habitat Partitioning. By W. Ronald Heyer. Pp. iii+27. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [121]
 Annual Survey Report: Ethical Pharmaceutical Industry Operations, 1975/1976. Pp. 35. (Washington, DC: Public Relations Dept., Pharmaceutical Manufacturers Association, 1155–15th St., N.W., 1976.) [131]
 Office de la Recherche Scientifique et Technique Outre-Mer, Paris et Bondy. Collection Travaux et Documents ORSTOM, No. 53: Communautés Rurales et Paysanneries Tropicales. Pp. 209. (Paris: ORSTOM, 1976.) 65 francs. [131]
 Deutscher Wetterdienst. Deutsches Meteorologisches Jahrbuch, Bundesrepublik, 1974. Pp. xxxvi+240. (Offenbach a.M.: Deutscher Wetterdienst, 1976.) [131]
 Sveriges Geologiska Undersökning. Avhandlingar och Uppsatser. Serie C. Nr. 720: Aitik-Geological Documentation of a Disseminated Copper Deposit—a Preliminary Investigation. By Hans Zweifel. Pp. 79. Nr. 721: Hydrogeologiska Förhållanden Inom Närke-Slättens Sedimentära Berggrund, med Fyra Plancher. Av Åke Möller, Per Engqvist och Carl-Fredrik Möller. Pp. 64. Nr. 722: Petrology of the Järnågsforsen

Tunnel, Western Medelpad, Central Sweden. By Lars Persson. Pp. 16. (Stockholm: Sveriges Geologiska Undersökning, 1976.) [141]
 World Health Organisation. International Non-proprietary Names (INN) for Pharmaceutical Substances, Cumulative List No. 4. Pp. xv+314. Geneva: WHO; London: HMSO, 1976. Sw. fr. 48; \$19.20. [171]
 World Directory of Schools for Medical Assistants/Répertoire Mondial des Ecoles d'Assistants Médicaux, 1973. Pp. 111. (Geneva: WHO; London: HMSO, 1976.) Sw. fr. 24; \$9.60. [171]
 New Zealand: Department of Health. Environmental Radioactivity—Annual Report 1975. Pp. 30. (Christchurch: National Radiation Laboratory, 1976.) [171]
 Energy, Mines and Resources Canada. Geological Survey of Canada. Paper 76–11: Geology of the Beaufort-Mackenzie Basin. By F. G. Young, D. W. Myhr and C. J. Yorath. Pp. 65. (Ottawa: Geological Survey of Canada, 1976.) \$6. [171]
 World Health Organisation. Technical Report Series, No. 598: Microbiological Aspects of Food Hygiene—Report of a WHO Expert Committee with the participation of FAO. Pp. 103. Sw. fr. 9; \$3.60. No. 599: Evaluation of Certain Food Additives—Twenty-second Report of the Joint FAO/WHO Expert Committee on Food Additives. Pp. 32. Sw. fr. 6; \$2.40. Guide to Sanitation in Tourist Establishments. By Joseph A. Salvato, Jr. Pp. 141. Sw. fr. 24; \$9.60. (Geneva: WHO; London: HMSO, 1976.) [171]
 United States Department of the Interior: Geological Survey. Water-Supply Paper 1817-F: Organic Carbon and Nitrogen Concentrations and Annual Organic Carbon Load of Six Selected Rivers of the United States. By Ronald L. Malcolm and Walton H. Durum. Pp. iv+21. (Washington, DC: US Government Printing Office, 1976.) [191]
 Le Noyau-Etoile de la Terre. Par Georges Dubou-dieu. Pp. 37. Essai d'Explication Optique des Points Chauds de la Terre. Conséquences. Par Georges Dubou-dieu. Pp. 55. Attempt at, and Implications of, an Optical Explanation for the Hot Spots of the Earth. By Georges Dubou-dieu. Pp. 54. (Meudon: Collège de France, Laboratoire de Géologie, 1975 and 1976.) [191]
 South African Institute for Medical Research. Annual Report for 1975. Pp. 84. (Johannesburg: South African Institute for Medical Research, 1976.) [191]
 A Right to Food: a Selection from Speeches by Adede H. Boerma, Director-General of FAO 1968–1975. Pp. 175. \$3.90p. The State of Food and Agriculture 1975 (FAO Agriculture Series, No. 1). Pp. xii+150. \$9.10. Impact Monitoring of Agricultural Pesticides. (Proceedings of the FAO/UNEP Expert Consultation on Impact Monitoring of Residues from the Use of Agricultural Pesticides in Developing Countries, held in Rome, 29 September to 3 October 1975.) Pp. iv+108. \$3.90. Report of the Eleventh Session of the Joint FAO/WHO Codex Alimentarius Commission, Rome, 29 March–9 April 1976. Pp. 99. \$3.57. (Rome: FAO, London: HMSO, 1976.) [201]
 Geological Survey of Western Australia. Annual Report for the year 1975. Pp. 144. History and Role of Government Geological Surveys in Australia. Edited by R. K. Johns. Pp. 111. (Perth: Geological Survey of Western Australia, 1976.) [201]
 United States Department of the Interior: Geological Survey. Professional Paper 990: Numerical Model of the Salt-Wedge Reach of the Duwamish River Estuary, King County, Washington. By Edmund A. Prych, W. L. Hauschild and J. D. Stoner. Pp. vi+34. Professional Paper 1006-A, B: Demand and Supply of Nonfuel Minerals and Materials for the United States Energy Industry, 1975–90—a Preliminary Report. By John P. Albers, et al. Pp. 56. (Washington, DC: US Government Printing Office, 1976.) [211]
 Cancer Detection and Prevention, Vol. 1, No. 1, 1976. Editor in Chief: Herbert E. Mirburgs. (Sponsored by The International Study Group for the Detection and Prevention of Cancer.) Pp. 1–239. Subscription rate for Volume 1 (1976), containing two issues: \$27.50 prepaid. Add \$2.30 per volume for postage outside the United States. (New York and Basel: Marcel Dekker, Inc., 1976.) [211]
 A Technical Report on Integrated Geophysical Studies in the Koyna Hydro-Electric Project Area of Maharashtra State India. By the National Geophysical Research Institute, Hyderabad, and Centre of Exploration Geophysics, Osmania University, Hyderabad. Edited by R. N. Athavale and Indra Mohan. Pp. xi+141. (Hyderabad: National Geophysical Research Institute, Council of Scientific and Industrial Research, 1976.) [211]
 Health: The Family Planning Factor. By Erik Eckholm and Kathleen Newland. (Worldwatch Paper No. 10.) Pp. 30. (Washington, DC: Worldwatch Institute, 1976 Massachusetts Avenue, NW., 1977.) \$2. [241]
 CERN—European Organisation for Nuclear Research. CERN 76–20: Proceedings of the 1976 CERN School of Physics, Wépion, near Namur, Belgium 6–19 1976. Pp. ix+129. CERN 75–22: Matrix Computation. By E. Edberg. Pp. vi+45. (Geneva: CERN, 1976.) [241]
 Smithsonian Contributions to the Earth Sciences, No. 18: A Catalog of the Type Specimens in the Mineral Collection, National Museum of Natural History. By Arthur Roe and John S. White, Jr. Pp. 43. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [241]
 US Department of the Interior: Geological Survey. Professional Paper 840: Descriptions and Analyses of Eight New USGS Rock Standards. Compiled and edited by F. J. Flanagan. Pp. vi+192. (Washington, DC: US Government Printing Office, 1976.) [241]
 United States Department of the Interior: Geological Survey. Bulletin 1426: Bibliography of Reports Resulting from US Geological Survey Technical Cooperation with Other Countries, 1967–74. By W. E. Bergquist. Pp. iv+68. (Washington: US Government Printing Office, 1976.) [251]

Interim Report of the National Research Council Committee of Nuclear and Alternative Energy Systems. (Assembly of Engineering—National Research Council.) Pp. 49. (Washington, DC: National Research Council, 2101 Constitution Avenue, NW, 1977.) [251]
 Publications du Centre National pour l'Exploitation des Océans (CNEXO) Série: Rapports Scientifiques et Techniques. No. 22: Pollution Chimique des Estuaires—Etat Actuel des Connaissances. Par J. M. Martin, M. Meybeck, F. Salvadori et A. Thomas. Pp. 286. No. 23: Etude des Courants et de la Marée à la Limite du Plateau Continental d'Après les Mesures Effectuées Pendant la Campagne "Golfe de Gascogne 1970". Par A. Cavanie et J. L. Hyacinthe. Pp. 39. No. 24: Circulation des Eaux d'Origine Méditerranéenne au Niveau du Cap St-Vincent—Hydrologie et Courants de Densité. Par F. Madelain. Pp. 73. No. 26: Etude Hydrologique et Variations Saisonnières dans le Proche Atlantique en 1973. Par B. Fruchaud-Laparra, J. Le Floch, J. Y. Le Tareau et A. Tanguy. Pp. 111. No. 27: Présentation des Données Recueillies Pendant la Campagne Overflow 73 à Bord du N/O Walther Herwig, 9 Août–4 Septembre 1973. Par F. Madelain. Pp. 30. No. 28: La Saliniculture Marine en Norvège état de Développement en 1975. Par Yves Harache. Pp. 149. No. 29: La Marégraphie au Large. Par J. L. Hyacinthe. Pp. 18. (Paris: Centre National pour l'Exploitation des Océans 1976.) [261]
 Sveriges Geologiska Undersökning, Stockholm. Lovö Geomagnetic Observatory—Year Book 1975. Pp. 36. (Stockholm: Sveriges Geologiska Undersökning 1976.) [261]
 United States Department of the Interior: Geological Survey. Professional Paper 881: Geologic Evaluation of Waste-Storage Potential in Selected Segments of the Mesozoic Aquifer System Below the Zone of Fresh Water, Atlantic Coastal Plain, North Carolina Through New Jersey. By Philip M. Brown and Marjorie S. Reid. Pp. iv+47+10 plates. Professional Paper 729-E: Late Quaternary Vegetation History of the Yellowstone Lake Basin, Wyoming. By Richard G. Baker. Pp. vi+48+10 plates. Professional Paper 792: Correlation of Late Cenozoic Tuffs in the Central Coast Ranges of California by Means of Trace—and Minor-Element Chemistry. By Andrei M. Sarna-Wojcicki. Pp. iv+30. Professional Paper 797: Icings Along the Trans-Alaska Pipeline Route. By Charles E. Sloan, Chester Zenone and Lawrence R. Mayo. Pp. vi+31. (Washington, DC: US Government Printing Office, 1976.) [261]
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 Bulletin of the American Museum of Natural History, Vol. 157, Article 6: Cranial Morphology of the European Jurassic Turtles *Portlandemys* and *Plesiochelys*. By Eugene S. Gaffney. Pp. 487–544. \$2.95. Vol. 158, Article 1: Revision of the Neotropical Cholineae, The Subgenus *Cholus* (*cholus*) (Coleoptera, Curculionidae). By Patricia Vaurie. Pp. 1–80. \$4.30. (New York: American Museum of Natural History, 1976.) [271]
 Annals of the Transvaal Museum, Vol. 30, No. 11: Neue Aphodinae aus Süd- und Ostafrika (Coleoptera: Scarabaeidae). Von Dr S. Endrödi. Pp. 109–118. Vol. 30, No. 12: A New Species of *Poecilimitis* Butler from the Drakensberg, Natal (Lepidoptera: Lycaenidae). By D. A. Swanepoel. Pp. 119–120+1 plate. Vol. 30, No. 13: South African Lepidoptera, 7. Descriptions and Notes on New Taxa of Rhopalocera. By L. Vari. Pp. 121–144+5 plates. (Pretoria: Transvaal Museum, 1976.) [271]
 Australian Journal of Zoology. Supplementary Series, No. 44: A Taxonomic Revision of the Anoplocephalidae, Cestoda: Cyclophyllidae) of Australian Marsupials. By L. Beveridge. Pp. 110. No. 45: A Revision of the Genus *Panpauxylla* Holland (Siphonaptaria: Pygospionyllidae). By D. K. Mardon. Pp. 69. (East Melbourne, Victoria: Editorial and Publications Service, CSIRO, 372 Albert Street, 1976.) [271]
 United States Department of the Interior: Geological Survey. Professional Paper 1011: The Environment of South Florida, a Summary Report. By B. F. McPherson and G. Y. Hendrix. Pp. vii+81. (Washington, DC: US Government Printing Office, 1976.) [271]
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 World Health Organisation. Technical Report Series, No. 594: WHO Expert Committee on Biological Standardisation—Twenty-seventh Report. Pp. 86. Sr. fr. 8; \$3.20. No. 600: New Trends and Approaches in the Delivery of Maternal and Child Care in Health Services—Sixth Report of the WHO Expert Committee on Maternal and Child Health. Pp. 98. Sr. fr. 12; \$4.80. (Geneva: WHO; London: HMSO, 1976.) [311]
 Australia: Commonwealth Scientific and Industrial Research Organisation. Report of the Division of Wild Life, 1974/1976. Pp. 600. (Melbourne: CSIRO, 1976.) [311]